THE UNIVERSITY OF MANITOBA

EVALUATION OF ACIDIC BUTANOL EXTRACTION AND ACIDIC METHANOL EXTRACTION FOR THE PREPARATION OF SUNFLOWER PROTEIN CONCENTRATES FREE OF DISCOLORATION.

by

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ABSTRACT

Removal of phenolic compounds from sunflower protein meal is essential for the preparation of a protein concentrates free of discoloration. The present study was undertaken to compare the effects of extraction of sunflower meals, from Sundak variety and Peredovik variety following a batch procedure or a countercurrent scheme, using two different solvent systems, acidic butanol (1-butanol - 0.005 N HCl, 92:8 v/v) or acidic methanol (methanol - 0.005 N HCl, 90:10 v/v).

Several criteria were used to evaluate the potential of acidic butanol or acidic methanol as solvents in preparing protein concentrates. These criteria were: 1) Efficiency in removing phenolics and 80% ethanol soluble sugars, 2) Protein losses, loss of lysine and isoleucine, 3) Protein denaturation and 4) Magnitude of discoloration. Both solvent systems were equally effective in removing phenolics. Acidic methanol was more effective in removing sugars than acidic butanol. Protein losses were similar regardless of the solvent system used. Protein quality was lower when acidic methanol was employed, as measured by protein denaturation. The quality of the concentrates in terms of color were similar as measured by a Hunterlab Color Difference Meter. Both solvent systems yielded an acceptable product.

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CHAPTER I

INTRODUCTION

Plant proteins are considered the most readily feasible alternative to supplement conventional sources of protein. So far, despite their high protein content, oilseed meals (excluding soybean) have been used only to a limited extent by the food industry. The reason for this is the presence of undesirable factors in the seeds. These factors include: high fiber content, toxic or antinutritional compounds and compounds which cause discoloration.

The problems involved in the use of oilseed proteins have been approached in different ways. Plant breeding has been used to reduce or eliminate the content of toxic compounds in the seeds. Specifically designed machinery permits more efficient removal of the pericarp from the seeds, thus, reducing the amount of fiber in the meals (Smith, 1969). Chemical methods have been successfully used to remove undesirable compounds from the oilseed meals, e.g. gossypol from cottonseed, glucosinolates from rapeseed. The importance of sunflower as an oilseed crop has risen significantly over recent years. Yields of up to 50% oil have been obtained through plant breeding and other genetic means (Panchenko, 1966). The protein content of sunflower seed is relatively

high, making the meal, after removal of the oil, a potentially good ingredient to improve the nutritional quality of other food products (Robertson, 1975). No toxic compounds have been found in sunflower meal (Singleton and Kratzer, 1969). Yet, the presence of relatively high concentrations of chlorogenic acid, and other phenolic compounds, have been identified as the major cause of discoloration of meal, protein concentrate and isolates (Cater et al, 1972). The development of green or brownish color prevents these products from being used to a large extent as ingredients or major constituents in foodstuffs. In addition to the color changes, phenolic compounds have been reported to reduce the availability of lysine (Davies et al, 1978), through binding with ϵ-amino group of lysine.

Many different methods have been proposed for the removal of phenolic compounds from sunflower meal. It would be desirable to obtain protein preparations with light color and negligible protein denaturation. A process which yields a product with such characteristics should result in minimal loss of protein. Most reported methods do not fulfill these requirements and need improvement.

Recently, a method using acidic butanol for the exhaustive removal of phenolics from sunflower meal has been reported by Sodini and Canella, 1977. These authors claimed that this procedure yielded a phenolic free concentrate with minimal protein denaturation. Gas producing oligosaccharides, e.g. raffinose, were partially removed. The resulting

protein concentrates were light in appearance. However no data regarding yields of the procedure were given.

The present study was undertaken to compare the effects of extraction of sunflower meals, from Sundak variety and Peredovik variety following the batch procedure of Sodini and Canella (1977), or a countercurrent scheme, using two different solvent systems, acidic butanol or acidic methanol. The effectiveness of the procedures and quality of the resulting products (protein, color) were evaluated.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Composition of Sunflower Seeds

Data on the composition of sunflower seeds have been reported by numerous researchers. Knowledge of the composition of the raw materials, in this case the sunflower seeds, provided the basis for the better utilization of this crop. Earle et al (1968) compared the composition of seven different varieties of sunflower seed. An inverse relationship between oil and protein content was observed. Oil content varied from 46.7 to 64.7% on a dry basis. Protein content ranged from 19.0 to 36.4% (N x 6.25) on a dry basis. Of the oil varieties analyzed differences in oil and protein values were considered not significant, since variability within varieties was as great as between varieties. On high oil sunflower hybrids, Robertson et al (1971) reported values for oil content from 28.8 to 44.7%. The crude protein ranged from 16.9 to 25.1%. Canella et al (1976) reported similar results on a study of Italian sunflower varieties. Dorrell (1976) observed a high correlation between chlorogenic acid content and oil in populations of North American sunflower seeds. Cultivated sunflower seeds contained from 1.1 to 4.5% chlorogenic acid (mean 2.8%). Dorrell (1976) also indicated that the concentration of chlorogenic acid of wild

sunflowers was higher for those specimens collected north of 40°N latitude, than for those collected south of 40°N latitude.

2.2 Composition of Sunflower Meals

The residue after oil extraction of sunflower seeds, called sunflower meal, constitutes an important by-product because of its high protein content and good amino acid balance (Clandinin, 1958). However, the presence of hull in the meal is undesirable as it increases the fiber content of the meal, consequently, reducing its nutritional value for monogastric animals (Clandinin, 1958). Hulls and chlorogenic acid in the meal cause undesirable discoloration of the meal, especially under alkaline conditions (Robertson, 1975). Clandinin (1958) indicated that a reasonable standard for a high quality sunflower seed meal should be: "not over 12% moisture, not over 10% crude fiber, not less than 40% crude protein and preferably less than 5% oil." Removal of the hull from the seeds, is important but is a difficult task, as Smith (1969) noted.

2.3 Sunflower Proteins

To facilitate their study proteins have been classified according to their solubility characteristics (Braverman, 1963). Although widely used, this classification is inappropriate (Haurowitz, 1963), as solubility of proteins depends not only on the concentration of salts,

but also on the pH, temperature and other factors. Osborne and Campbell (1897) reported the separation of the "proteids" of sunflower seed for the first time, by solubilizing them with sodium chloride. From their findings they concluded that the most abundant "proteid" consisted of a single globulin. A slight modification of Osborne's protein fractionation, was used by Sosulski and Bakal (1969) and Gheyasuddin et al (1970) for the fractionation of sunflower meal proteins. The mean values reported were: water soluble (albumins), 20%; salt soluble (globulins), 56%; alcohol soluble (prolamins), 3%; dilute alkali soluble (glutelins), 15%; and residue 6%. Similar results were reported by Mosse and Baudet (1972), Krasil'nikov et al (1974) and Shehebakov et al (1971). The differences observed were probably due to sample and/or varietal differences.

Characterization of the salt-extractable proteins from sunflower meal from three different varieties was carried out by gel chromatography, electrophoresis and amino acid composition (Sabir et al, 1973). Five fractions were separated by gel chromatography on Sephadex G-200, the molecular weights ranged from 600,000 for the heaviest fraction to less than 5,000 for the lightest fraction. The five fractions were similar in molecular weight to those of soybean proteins. Joubert (1955) used sedimentation and diffussion methods and determined molecular weights of 343,000

and 19,000 for the two major protein components of sunflower meal. Mosse and Baudet (1972) found that the albumin fraction of sunflower seed proteins contained 6.7% lysine, while globulin, the major protein constituent contained only 3% lysine. This accounts for the lysine deficiency of sunflower meal, protein concentrates and isolates reported by several authors (Clandinin, 1958; Earle et al, 1968, and Evans and Bandemer, 1967).

Sunflower proteins appear to be 90% digestible with a biological value of 60%. This is comparable to soybean meal proteins. (Clandinin, 1958)

Earle et al (1968) found no significant differences in the amino acid composition between different sunflower varieties. Recovery of nitrogen plus ammonia was greater than for most other oilseed meals. Sunflower proteins contained 34.1% of essential amino acids, which is below that of animal products, but similar to most plant seed proteins. Chemical analysis indicated adequacy of essential amino acids, except for lysine and isoleucine. The chemical score for sunflower proteins for human nutrition as determined by FAO (1965) was 89 (whole egg is 100). Similar results for the nutritional value of sunflower proteins were reported by Clandinin (1958) and Pustrovoit et al (1972).

Amino acid analyses of sunflower seeds by Evans and Bandemer (1967), showed deficiencies of lysine and

methionine. Supplementation of sunflower meals with lysine and methionine increased the protein value from 59 to 85.

2.4 Effect of preparation methods for sunflower protein products on protein quality.

Protein denaturation effects of sunflower seeds extraction procedures were studied by Gurdevand and Marinchevski (1967). More denaturation was observed during evaporation and drying in a battery type extraction system (16-24%), than during continuous extraction (9%). Similar observations were reported by Stoyanov et al (1967). Sarwar et al (1973) reported PER values for sunflower proteins equal to those of casein. They observed significantly lower weight gains in mice fed sunflower protein isolates, than when fed the parent meals. This indicated that protein isolation procedures decreased the protein quality.

Destruction of 18% of the lysine, 25% of the tryptophan and 11.6% of the methionine resulted when sunflower seeds were heated at 121°C for 15 minutes in an autoclave (Stopajauljevic et al, 1971). The biological value decreased on the average 11.7%. Similar effects were observed by Bandemer and Evans (1963) and Morrison et al (1953). On the other hand, Basualdo et al (1972) found the nutritional value of sunflower meal (measured as essential amino acids, available lysine, NPU and digestibility) was not significantly impaired by processing. However they observed a decrease in the amount of lysine during storage.

Most of the methods commonly used for preparation of protein isolates require alkaline solubilization, followed by acid precipitation of the proteins, in addition, high temperatures are sometimes used to enhance alkaline extraction. Murray et al (1978) pointed out the effects of such treatments on proteins, e.g. formation of lysinoalanine (LAL), which may be toxic. In addition LAL decreases the protein efficiency ratio (PER). Considering the potential problems involved in the alkali/acid processing, Murray et al (1978) developed a method for the preparation of protein isolates under mild conditions of pH and temperature, using the principle of salting in. The proteins were salted in with common grade salts at near neutral pH and then precipitated by a phenomenon referred to as "hydrophobic-out". This method can be applied to a variety of protein sources, including sunflower, and it largely preserves the chemical and physical characteristics of the proteins.

In preparing protein isolates from sunflower seeds it is important to choose a method which will remove phenolic compounds from the isolates. In general this is difficult as phenolics are usually covalently attached to proteins.

Considering that sunflower proteins may also be used in the form of meals or protein concentrates it is important to consider the effect that the preparation of these products can have on nutritional and functional properties of the protein.

2.5 Determination of protein denaturation

Decrease in nitrogen solubility over a certain range of pH (Nitrogen Solubility Profile) or on a certain pH (Nitrogen Solubility Index, NSI) have been widely used as an indication of protein denaturation (Smith and Johnson, 1948; Sosulski and Bakal, 1969; Gheyasuddin et al, 1970; Kilara et al, 1972; Cater et al, 1972).

Recently, Differential Scanning Calorimetry (DSC) has been utilized to measure the degree of denaturation of proteins in terms of the heat of transition (ΔH) (Karmas and Di Marco, 1970; Delbeu and Crescenzi, 1969; Donovan and Beardslee, 1975).

In an earlier study Steim (1965), using Diferential Thermal Analysis DTA, showed the feasibility of thermal analysis for the study of denaturation of proteins in solution. The basis of the DTA and DSC measuring systems is to compare the rate of heat flow to the sample and an inert reference material. These are heated or cooled at the same rate. Events in the sample such as phase transitions or chemical reactions which involve absorption or evolution of heat cause a change in the differential heat flow. In a plot of differential heat flow against temperature or time the event is recorded as a peak. The area under the curve gives the heat change (ΔH) associated with the event and the direction of the peak indicates whether the heat flow is exothermic or endothermic (Ladbrooke, 1977).

Privalov and Khechinashvili (1974) obtained calorimetric data for five single chain proteins for which full structures are known. Their principal findings were:

1. Δ Cp (change in specific heat) does not vary with temperature but the numerical value is different for each protein.

2. ΔH (heat change) varies linearly with temperature and the slope of the line $\frac{\delta \Delta H}{\delta \Delta T}$ is equal to Cp. In other words the transition obeys Kirchoffs Law which means that ΔH depends solely on temperature.

3. There is a linear correlation between $\frac{d\Delta H}{dT}$ and the number of contacts between pairs of non-polar groups for each protein.

4. The number of hydrogen bonds formed by each protein were also calculated and all were found to be essentially the same.

The data provided by these authors indicate the feasibility of DSC for the measurement of the degree of protein denaturation.

2.6 Carbohydrates in Sunflower seed meals

The carbohydrates in sunflower seeds, meals and concentrates have been investigated by several workers (Mikolajczak et al, 1970, Sabir et al, 1975 and Cegla and Bell, 1977). Defatted sunflower meals contained small amounts of monosaccharides (0.6% on dB) which were

identified as arabinose, fructose and glucose. The major proportion of the 80% ethanol soluble sugars (7-10% of total solids) were oligosaccharides, sucrose (4%) and raffinose (2%). Other oligosaccharides identified were trehalose, maltose, melibiose and stachyose. Polysaccharides found in sunflower meal included to be small amounts of pentoses, hemicelluloses and cellulose (Sabir et al, 1975).

Of some importance is the occurrence of raffinose and stachyose. Rackis et al (1970) found, in a study with soybean, that these oligosaccharides were related to gas production by intestinal bacteria. These sugars should therefore be removed from sunflower protein preparations. Simple sugars are involved in browning reactions, which in some instances are undesirable. Removal of these sugars may therefore result in sunflower preparations of lighter appearance.

Functional properties of sunflower meals and concentrates (e.g. water holding capacity, gelation, etc.) could be influenced by polysaccharides however no investigations have been reported on the effects of these compounds on the properties of sunflower protein preparations.

2.7 Phenolic Compounds in Sunflower Seed Meals

Phenolic compounds, including chlorogenic acid, are widely distributed in the plant kingdom. Chlorogenic acid or 3-caffeoylquinic acid is formed by esterification of caffeic acid with quinic acid (Ribereau-Gayon, 1972).

The structure of these acids, namely the presence of the phenolic groups, make them reactive compounds. Disease resistance in plants has been related to phenolic compounds. Van Sumere et al (1975) attributed this role of phenolic acids to the quinones which are very reactive oxidation products of phenolics. Phenolic acids have other roles in plants. For example they are precussors of lignin and thus it would be impossible to breed plants free of phenolic acids.

Osborne and Campbell (1897) reported the presence in sunflower meal of what they called helianthotannic acid, later identified as chlorogenic acid (Gorter, 1909). Other phenolic compounds have been identified in sunflower meal. Milic et al (1968) isolated and identified chlorogenic acid and quinic acid. Mikolajczak et al (1970) identified caffeic acid, chlorogenic acid, 3,5-dicaffeoylquinic acid, a disubstituted cinamic acid and also a monoester of quinic acid.

Sabir et al (1974) reported the identification of eight different phenolic compounds: chlorogenic acid, isochlorogenic acid, hydroxycinnamic acid-sugar ester, caffeic acid, isoferulic and sinapic acid like, p-coumaric acid like and two unknown compounds. Chlorogenic acid and related compounds constituted about 70% of the total phenolic compounds. In a subsequent study Sabir et al (1975) found soluble chlorogenic acid associated with low molecular weight polypeptides and oligonucleotides.

The levels of phenolic acids in sunflower seeds and meals were determined by several investigators (Milic et al, 1968; Mikolajczak et al, 1970; Brummett and Burns, 1972; Sabir et al, 1974a; Felice et al, 1976). Their findings can be summarized as follows: sunflower meal contains 1.9 to 2.5% total phenolic acids of which 70-80% is chlorogenic acid and 20-30% caffeic acid, and small amounts of the others.

2.8 Reaction of phenolic compounds with proteins and amino acids

Phenolics combine with proteins reversibly by hydrogen bonding and irreversibly by oxidation followed by covalent condensation. Loomis and Battaile (1966) found that the oxidation of phenolic compounds leads to formation of highly reactive quinones, which can polymerize. These polymers react in a similar manner to the monomers in the presence of proteins. Pierpoint (1969) reported similar findings and studied the reaction of quinones with amino acids. Amino acids, excluding lysine and cysteine, react with quinones primarily through their -amino groups to give red or brown products. These reactions, which compete with the polymerization of quinones, are followed by secondary reactions. These secondary reactions may absorb oxygen and give products with other colors. The -amino group of lysine reacts with the O-quinones in a similar fashion. The

thiol group of cysteine reacts with the quinones without absorbing oxygen, giving colorless products.

The reaction of quinones with lysine and the subsequent polymerization of phenolics into tannin-protein complexes could render a large number of amino acids inaccessible to the digestive processes of monogastric animals. A small amount of oxidation can cause a large diminution in nutritional value (Allison, 1971; Davies et al, 1978). Dryden and Satterlee (1978) reported that chlorogenic acid bound to a casein model system, caused a small, but real drop in the "in vitro" protein digestibility of the casein complex and a significant inhibition in the growth of Tethrahymena pyriformis W, resulting in a low Tetrahymena based PER.

2.9 Removal of phenolics from sunflower meal

Osborne and Campbell (1897) and Smith and Johnsen (1948), used hot ethanol (70%) to extract the chlorogenic acid from sunflower meal. Total removal was not achieved by these methods. In addition severe protein denaturation was observed. Joubert (1955) attempted to remove chlorogenic acid at room temperature by extracting with 50% ethanol followed by acetone washing. Joubert (1955) however, did not report on the degree of protein denaturation and the color of the preparations.

Alkaline protein solubilization (pH 10.5) in the

presence of 0.25% sodium sulfite followed by acid precipitation was used by Gheyasuddin et al (1970). Further extraction with 50% isopropanol yielded an isolate that showed little protein denaturation (nitrogen solubility profile), but developed brown color at alkaline pH. Using the principle of the diffusion of low molecular weight compounds through semipermeable membranes, Sosulski et al (1972, 1973) developed a batch and a continuous diffusion method to remove the chlorogenic acid from sunflower kernels. Water at ratios of 10:1, 20:1, with final ratios of 600:1 to 80:1 for the continuous process, temperature of 20, 40, 60 and 80°C and pH range 2.3 to 9.5 were used for the extraction, 75% ethanol was used for comparison. The main drawbacks of the batch diffusion were: long extraction periods, high protein denaturation and the large volumes of water required. The continuous diffusion was developed to try to overcome these problems, however extraction periods continued to be long (4 hrs.) and high solid losses were observed. Protein denaturation was lower in the continuous process. Based on these findings, Fan et al (1976) reported the removal of about 90% of the chlorogenic acid of sunflower flour using water, acid or alcohol in a countercurrent process. The resulting protein concentrates contained over 70% protein and were light in color under alkaline pH conditions. The countercurrent system was more efficient in solvent use and chlorogenic acid removal than batch extraction. However

it resulted in high losses of solids (40%) and proteins (25%)

Sodini and Canella (1977) claimed effective removal of color-forming phenolics (chlorogenic and caffeic acids) by exhaustive extraction with acidic butanol. Oligosaccharides, such as sucrose, were removed efficiently by this method. Negligible protein denaturation was observed (as determined by nitrogen solubility profiles of the prepared protein isolates). No data on yields of the method were provided.

2.10 Potential applications of sunflower protein concentrates and isolates

At the present time confectionery sunflower seeds are available for human consumption, but sunflower protein concentrates and/or isolates are not used in foods on a commercial scale. However, many potential applications for sunflower protein preparations have been proposed.

Diffusion extracted sunflower concentrates were added to commercial wiener mixes to increase the protein content from 12% in the control to 14% in the protein-supplemented wieners. Soy flour and concentrate were used for comparison. Shrinkage, color, peelability, firmness, cooking properties and sensoric characteristics were judged in the processed wieners. Wieners containing sunflower concentrates were as acceptable as wieners which contained soy flour or soy concentrate, although both products were rated lower than the all meat product in sensory properties. An unacceptable

product resulted when sunflower flour replaced sunflower protein concentrates (Lin et al, 1975). Incorporation of sunflower protein concentrates into milk blends were evaluated by Fleming and Sosulski (1977). Removal of color producing phenolics reduced the nitrogen solubility of sunflower protein concentrates. But heat, mechanical agitation and emulsifiers were effective in resolubilizing more than 80% of the nitrogen. An equal blend of sunflower concentrate and milk had an equivalent chemical score to a soy milk blend, but the color and flavor profile was low.

Bread has also been supplemented with sunflower protein concentrates (Patt et al, 1974; Jain et al, 1975; and Fleming and Sosulski, 1977), and assessed by chemical, physical and sensory parameters. In general such formula alterations yielded acceptable bread products.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Sunflower seeds of two varieties were used in this study; a confectionery variety, Sundak and an oil variety, Peredovik. The proximate composition of the samples is given in Table 1. Both samples were obtained from Northern Sales Co. Ltd., Winnipeg, and were of 1977 crop.

Table 1. Proximate Composition of Sunflower Kernels

Constituents	VARIETY	
	Peredovik	Sundak
	(% dB)	(% dB)
Oil	64.10	57.08
Protein (N x 6.25)	23.23	30.97
Ash	3.07	3.39
Carbohydrates[a]	4.87	5.65
Phenolics[b]	1.80	1.91

[a]Soluble in 80% ethanol, as glucose

[b]as chlorogenic acid

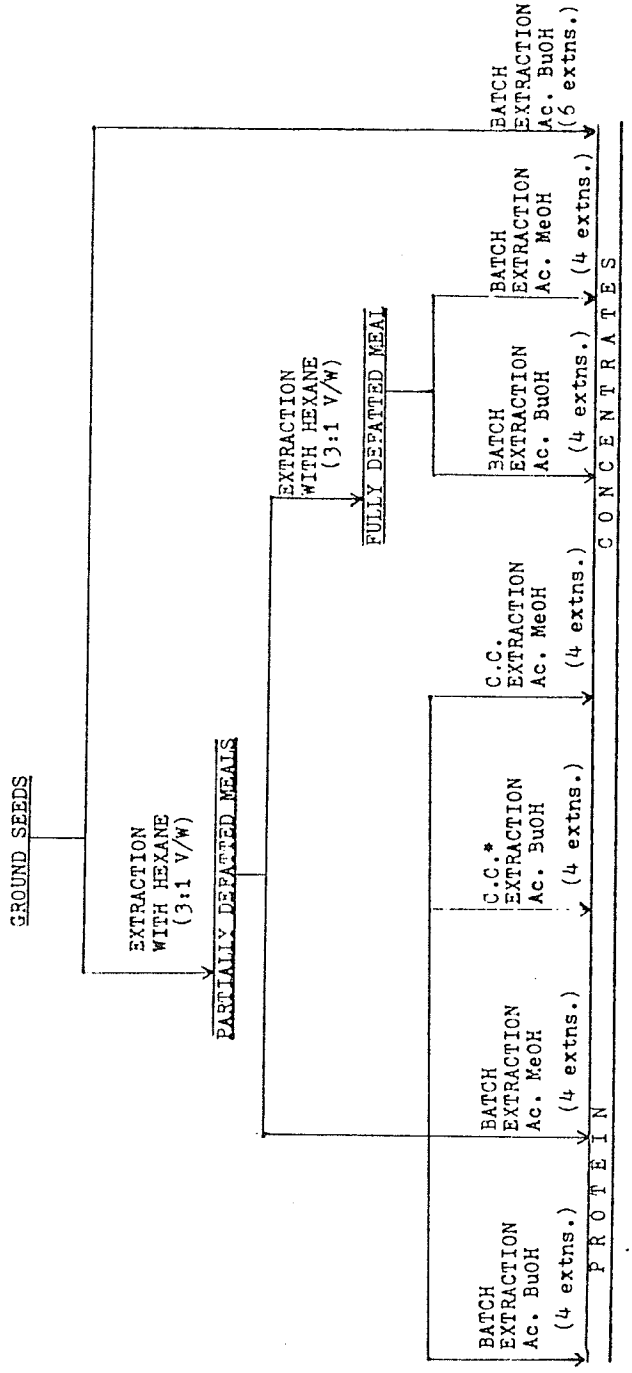
3.2 Preparation of protein concentrates

Protein concentrates were prepared by three different ways, as shown in Fig. 1.

3.2.1 Removal of oil

The dehulled seeds were ground in hexane at a solvent to sample ratio of 3:1 (v/w), using a Waring blender for two

Figure 1. Preparation of Protein Concentrate



* c.c. = Countercurrent

minutes at medium speed. The extraction was continued at room temperature for 16 hours, by shaking the solvent with the ground seeds on a mechanical shaker. After extraction the meals were separated by filtration through Whatman paper No. 4, under suction. The meals were desolventized by evaporating the remaining solvent at room temperature, under normal pressure, for two days. This procedure yielded partially defatted meals. One further extraction with hexane, under the same conditions, was required to yield fully defatted meals.

3.2.2 Batch procedure

For the preparation of protein concentrates samples from partially defatted meal, the meal was suspended in 1-Butanol-0.005N HCI (92:8, v/v) or Methanol-0.005N HCI (90:10, v/v), at a solvent to sample ratio of 20:1. The pH was adjusted to 5.0 with 0.5N HCI, and kept constant throughout the extraction by addition of 0.5N HCI. The extraction was carried out at room temperature for 15 minutes, with magnetic stirring. The suspension was filtered through Whatman No. 3 paper under suction. The residue was extracted three additional times under identical conditions (Sodini and Canella, 1977). Protein concentrates were also obtained by extracting ground seeds six times with acidic butanol, under the same conditions as were used for extracting partially defatted meal.

3.2.3 Countercurrent procedure

This procedure was carried out according to Fan et al. (1976). The conditions of pH, temperature, solvent to sample ratio and time of extraction were the same as for the batch procedure.

3.3 Proximate Analysis

Determination of moisture, ash and fat were carried out according to A.O.A.C. (1975). Nitrogen determinations were by the boric acid modification (A.A.C.C., 1962) of the A.O.A.C. (1960) Kjeldahl procedure for total nitrogen, except that the mercuric oxide and potassium sulfate were replaced by 10g of a premixed catalyst (kel-pak No. 2, Curtin Matheson Scientific Inc.). Protein content was reported as N x 6.25.

3.4 Osborne Fractionation

Two gram samples of partially defatted meal were successively extracted with the following solvents: distilled water, 5% NaCl, 70% ethanol (at 65°C) and 0.2% NaOH (Sosulski and Bakal, 1969). The samples were extracted 15 minutes with each solvent, then centrifuged at 1200 x g for 15 minutes. The supernatant was collected for Kjeldahl nitrogen determinations.

3.5 Amino acid analysis

Amino acid analyses were carried out on a Beckman 119c analyzer. The Beckman modification of the single column procedure of Spackman et al. (1958) was applied. The sulfur

containing amino acids, methionine and cystine were determined according to Hirs (1967).[1]

3.6 Determination of Sugars

Sugars were extracted from samples by refluxing twice for 30 minutes with 80% ethanol (solvent to sample ratio 50:1, v/w). The samples were centrifuged (4000 x g for 10 minutes), the supernatants pooled. Soluble sugars were determined by the phenol-sulfuric acid method (Dubois et al, 1956). The absorbance of the samples was measured with a Unicam SP600 spectrophotometer, at 488 nm. The concentration of sugars was calculated from a calibration curve using glucose as the standard.

3.7 Determination of Phenolics

Phenolics in samples were determined according to the method described by Dorrell (1976). The absorbance of extracts was measured with a Unicam SP800B spectrophotometer at 329 nm. The concentration of phenolics was calculated from a calibration curve using chlorogenic acid as standard.

3.8 Nitrogen Solubility Profile

One gram samples were extracted with 50 ml of distilled water for 20 minutes, the pH range was from 2 to 9, and was adjusted before extraction and kept constant by addition of either 0.5N NaOH or 0.5N HCI. Samples were then centrifuged at 4000 x g for 15 minutes, and Kjeldahl nitrogen

[1]These analyses were performed by Mr. P. Mills of the Dept. of Animal Science.

determined in the supernatants.

3.9 Nitrogen Solubility Index (at pH 7.0)

This determination was carried out by extracting with distilled water at pH 7.0, under the same conditions as for the nitrogen solubility profile.

3.10 Differential Scanning Calorimetry (DSC)

The DSC analyses of the meal and protein concentrates were performed with a DuPont Differential Scanning Calorimeter model 910. These analyses were performed by Mr. T. J. Maurice, Research Dept., General Foods Ltd., Cobourg, Ont.

3.11 Color Measurement

3.11.1 On the dry samples

The dried samples were ground to a fine powder in a porcelain mortar before color measurements were taken. Color measurements were taken using a Hunterlab Model D25 Color Difference Meter. Determinations were made in triplicate. The white tile, Hunterlab standard No. D25-1333, with the tristimulus values L = 93.8, a - -1.1, and b = 2,3, was used as standard. Total color difference was described as:

$$\Delta E = \left[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\right]^{1/2}$$

in comparison to the white standard.

3.11.2 Color measurement on slurries

Color measurements were taken on 16% flour in water slurries, following a slightly modified procedure of Hoehn et al (1976). The slurries were prepared as outlined in Table 2. Heating was for 90 sec. in a microwave oven (Varian Industrial Systems Magnetron, Model PPS-2.5 A, and Gerling-Moore microwave cavity) with intermittent stirring. The pH of the slurries was adjusted by addition of 0.5N NaOH.

Table 2. Preparation of Slurries for Color Measurement

Treatment	Treatment of slurries for color measurement
1	Slurry 16% (w/v), no further treatment
2	Slurry 16% (w/v), heated up to 80°C.
3	Slurry 16% (w/v), pH adjusted to 8.0
4	Slurry 16% (w/v), pH adjusted to 8.0, heated up to 80°C.

3.12 Statistical Analysis

A one way analysis of variance was carried out to assess color differences among samples. Multiple regression analyses were applied to L or ΔE values as dependent variables, and concentration of phenolics, concentration of sugars, ΔH and Nitrogen solubility index as independent variables to determine the relationship between color and these factors. Coefficients of determination were calculated to measure the strength of relationship between color (Hunter Values L, a and ΔE) and concentration of phenolics.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characterization of Raw Materials

The two selected sunflower varieties represented an oil type and a confectionery type. As expected the oil variety (Peredovik) had a higher oil content and a lower protein content than the confectionery variety (Sundak) as shown in table 1 (Section 3.1). The inverse relationship between oil and protein content has been well documented and agrees with findings of other authors (Earle et al, 1968). The differences in the concentrations of other constituents found in these two varieties were of less magnitude. This is illustrated by the slightly higher values for soluble sugars and phenolics were obtained for Sundak variety compared to Peredovik variety. Both the amount of soluble sugars and the amount of phenolics were in agreement with reported values for these constituents (Mikolajczak et al, 1970; Sabir et al, 1974, 1975; Cegla and Bell, 1977).

A further attempt to characterize these two varieties was performed by Osborne classification of the proteins. The results are presented in Table 3. The major proteins of both varieties were primarily salt soluble, globulins, also contained significant amounts of water soluble, albumins, and alkali soluble, glutelins. This data is in agreement with those presented

Table 3

Osborne Fractionation of the Proteins of the Sunflower Kernels

	Percent of total meal nitrogen soluble in			
Variety	H_2O	5% Nacl	70% EtOH	0.2% NaOH
Sundak	14.82	56.07	3.45	16.06
Peredovik	21.92	59.37	4.15	12.77

by Sosulski and Bakal (1969). The proportion of water soluble albumins and ethanol soluble prolamins proteins was higher for the samples of Peredovik variety. In general the proteins of the Peredovik variety showed a higher solubility than those of Sundak, with the exception of the glutelins of Sundak which were more soluble.

The amino acid composition of both varieties is given in Table 4. There were no major differences found between the two varieties. This was consistent with studies by Earle et al, (1968) who reported similarity of amino acid composition for a selected group of seven different sunflower varieties.

4.2 Efficiency of Extraction Procedures

Phenolic, sugar and protein content were determined in all the meals and protein concentrates, as shown in Appendix 1. Amino acid composition was determined in the meals (Table 4) and in the protein concentrates (Appendixes 2,3,4). The protein content of the concentrates ranged from 59 to 67%. Yields based on dehulled seeds were 34 to 48% total solids for the Sundak variety and 26.4 to 40% total solids for Peredovik variety. The slightly lower yields for Peredovik variety than for Sundak variety reflect the higher protein content of the Sundak variety. Yields in terms of protein were between 76.6 to 92% with a mean value of 86.8%.

Efficiencies of the extraction procedures were assessed. These were based on the removal of phenolics and 80% ethanol soluble sugars. Further evaluation of the extraction

procedures were based on protein loss, lysine loss and isoleucine loss.

Removal of phenolics and sugars, and loss of protein, lysine and isoleucine were calculated. The calculations were based on the composition data of the samples given in Appendix 1. An example of the computations is given in Appendix 5. The results obtained for both varieties and the different extraction procedures are compiled in Table 5. Removal of phenolics from partially or fully defatted meals was better than 90% for both solvent systems (acidic butanol and acidic methanol) and all extraction procedures. Acidic methanol was a more effective solvent for the removal of sugars than acidic butanol. On the average acidic methanol solubilized 10-20% more of the sugars than acidic butanol. Efficiency of acidic butanol in terms of removal of phenolics and sugars was in agreement with data reported by Sodini and Canella (1977).

The values obtained for protein loss, lysine loss and isoleucine loss are shown in Table 5. Protein loss was smaller with acidic butanol than with acidic methanol for both extraction procedures (batch and countercurrent). Lysine loss and isoleucine loss followed similar trends as protein loss. For both amino acids the decreases were of same magnitudes (18%) but were on the average about 5% points higher than those of protein.

Table 5

Effectiveness of Extraction Procedure

Variety	Procedure and Solvent	Phenolics removed as Chlorogenic acid (%)	Sugars removed soluble in 80% EtOH (%)	Protein Loss %	Lysine Loss %	Isoleucine Loss %
SUNDAK	Partially Defatted with Hexane (Control)	0.0	0.0	0.0	0.0	0.0
	Batch. Ac. Butanol	96.2	78.3	10.4	17.0	21.8
	Batch Ac. Methanol	92.1	98.3	16.7	24.3	23.6
	Countercurrent. Ac.BuOH	90.1	78.3	8.2	12.9	12.1
	Countercurrent. Ac.MeOH	92.2	95.8	9.2	12.4	5.6
	Fully Defatted with Hexane (Control)	0.0	0.0	0		
	Batch. Ac. Butanol	91.5	78.4	8.7		
	Batch. Ac. Methanol	97.5	98.9	13.8		
	Directly extracted with BuOH (Batch)	99.5	86.8	27.6	21.1	19.6
PEREDOVIK	Partially Defatted with Hexane (Control)	0.0	0.0	0.0		
	Batch. Ac. Butanol	98.0	88.4	10.4	6.7	0.3
	Batch. Ac. Methanol	96.7	98.4	13.1	23.0	24.2
	Countercurrent Ac.BuOH	92.8	81.1	9.3	13.7	13.8
	Countercurrent Ac.MeOH	96.5	98.4	13.4	25.3	23.1
	Fully defatted with Hexane (Control)	0.0	0.0	0.0		
	Batch. Ac. Butanol	96.4	86.1	8.10		
	Batch. Ac. Methanol	98.2	99.1	11.70		
	Directly extracted with BuOH (Batch)	99.5	92.0	23.40	29.7	30.0

4.3 Effect of Extraction Procedures on Protein Denaturation

The magnitude of protein denaturation determines the quality of proteins to a high degree. In general a highly native protein or a protein with little denaturation shows better functionality than a completely denaturated protein (Murray et al, 1978). Partial denaturation of proteins can however improve their nutritional value. One example would be soybeans, mild heat treatments seem to improve the digestibility of its protein. This is related to denaturation of a trypsin inhibitor. Since sunflower protein does not contain any antinutritional factors one should aim for protein preparation with the least amount of denaturation. Thus the measurement of protein denaturation is a measurement of protein quality.

The parameters measured in this study to estimate protein denaturation were the following:

a) Nitrogen solubility index at pH 7.0 (NSI)

b) Transition enthalpy (ΔH) obtained by DSC

c) Nitrogen solubility profiles over a pH range from pH 2 to 9.

NSI and ΔH values are presented in table 6. The obtained data for the partially and fully defatted meals confirmed that Peredovik protein was more soluble than Sundak protein. Fan et al, (1976) and others reported similar NSI values for defatted sunflower meals. To estimate the effect of extraction procedures on protein denaturation NSI and ΔH values were

Table 6

Effect of Extraction Procedure on the Proteins

Variety	Procedure and Solvent	Nitrogen (%) Recovered at pH 7.0 a	ΔH	Variety	Procedure and Solvent	Nitrogen Recovered at pH 7.0 (%)b	ΔH
SUNDAK	Partially Defatted Hexane (Control)	36.55	4.46	PEREDOVIK	Partially Defatted meal (control)	54.53	4.24
	Batch. Butanol	24.11	2.22		Batch. Butanol	42.73	2.82
	Batch. Methanol	23.40	2.02		Batch. Methanol	27.73	1.94
	Countercurrent Butanol	21.60	1.33		Countercurrent Butanol	38.00	1.94
	Countercurrent Methanol	15.48	0.50		Countercurrent Methanol	21.42	0.58
	Fully defatted Hexane (Control)	37.58	4.39		Fully defatted meal (control)	56.44	3.83
	Batch. Butanol	21.94	1.26		Batch. Butanol	34.65	2.76
	Batch. Methanol	13.92	0.10		Batch. Methanol	27.66	1.68
	Directly extracted Butanol	25.22	1.49		Directly extracted with Butanol	30.95	2.93

[a] Value for ground seeds = 36.2

[b] Value for ground seeds = 64.5

compared with NSI and ΔH values of the parent partially defatted meals and the parent fully defatted meals respectively. The findings can be summarized as follows.

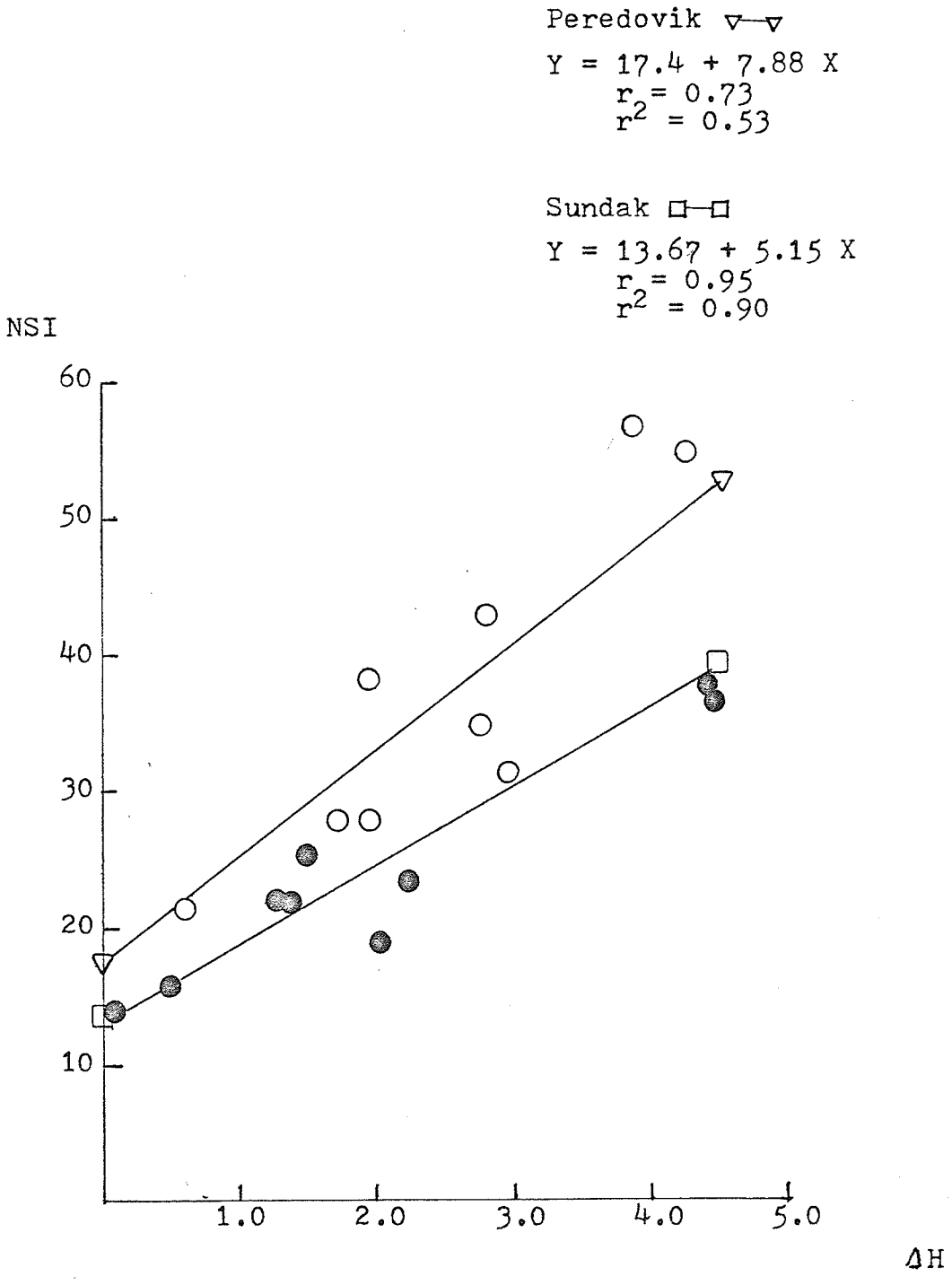
Samples extracted with acidic butanol (batch or countercurrent procedure) had higher NSI and ΔH values than those extracted with acidic methanol. The countercurrent procedure caused more protein denaturation than the batch procedure. This agreed with findings reported by Fan et al (1976). They reported that the countercurrent scheme compared with the batch extraction system caused a higher degree of denaturation.

Direct extraction of crushed sunflower kernels with acidic butanol resulted in protein concentrates with higher degrees of protein denaturation when compared with the ones prepared from defatted meals. The crushed kernels were extracted for six times to ensure complete removal of oil. The defatted meals were extracted four times with acidic butanol resulting in slightly better quality of the proteins.

NSI and ΔH values indicated similar trends and were correlated as shown in Figure 2. A linear correlation coefficient of $r = 0.95$ ($p < 0.001$) was found for the Sundak variety. In the case of Peredovik the correlation coefficient r was 0.73 ($p < 0.025$).

The nitrogen solubility profiles as presented in Figures 3 to 9 confirmed the findings obtained by measuring NSI or ΔH. All extraction procedures resulted in a slight decrease of the protein solubility over the entire pH range

Figure 2. Correlation between values of N.S.I. and ΔH



investigated. However no other changes were observed. The isoelectric point was between pH 4.5 and 5 for all samples.

Both varieties (Sundak and Peredovik) followed similar solubility patterns (Figure 3). The overall protein solubility was higher for Peredovik variety. Extraction with acidic methanol resulted in lower overall solubility than with acidic butanol (Figures 4 and 7), this was independent of the variety.

Comparison of batch and countercurrent procedures showed the following. The nitrogen solubility profiles were similar for both varieties and both procedures when acidic butanol was used as solvent, (Figures 5 and 8).

Extraction with acidic methanol in the countercurrent scheme for Sundak variety resulted in a decrease in solubility as compared to the batch extraction (Figure 6). The samples of Peredovik variety extracted with acidic methanol had similar nitrogen solubility profiles for the batch or countercurrent procedure (Figure 9).

4.4 Color Measurements on Sunflower Meals and Concentrates

Discoloration of sunflower meals and concentrates has been the major drawback for the acceptance of products containing sunflower protein preparations. A protein preparation with a light color, ideally white, would be the characteristic of a high quality product. In this study color of meals and concentrates was evaluated using a Hunter color difference

Figure 3 Nitrogen solubility of partially defatted meals

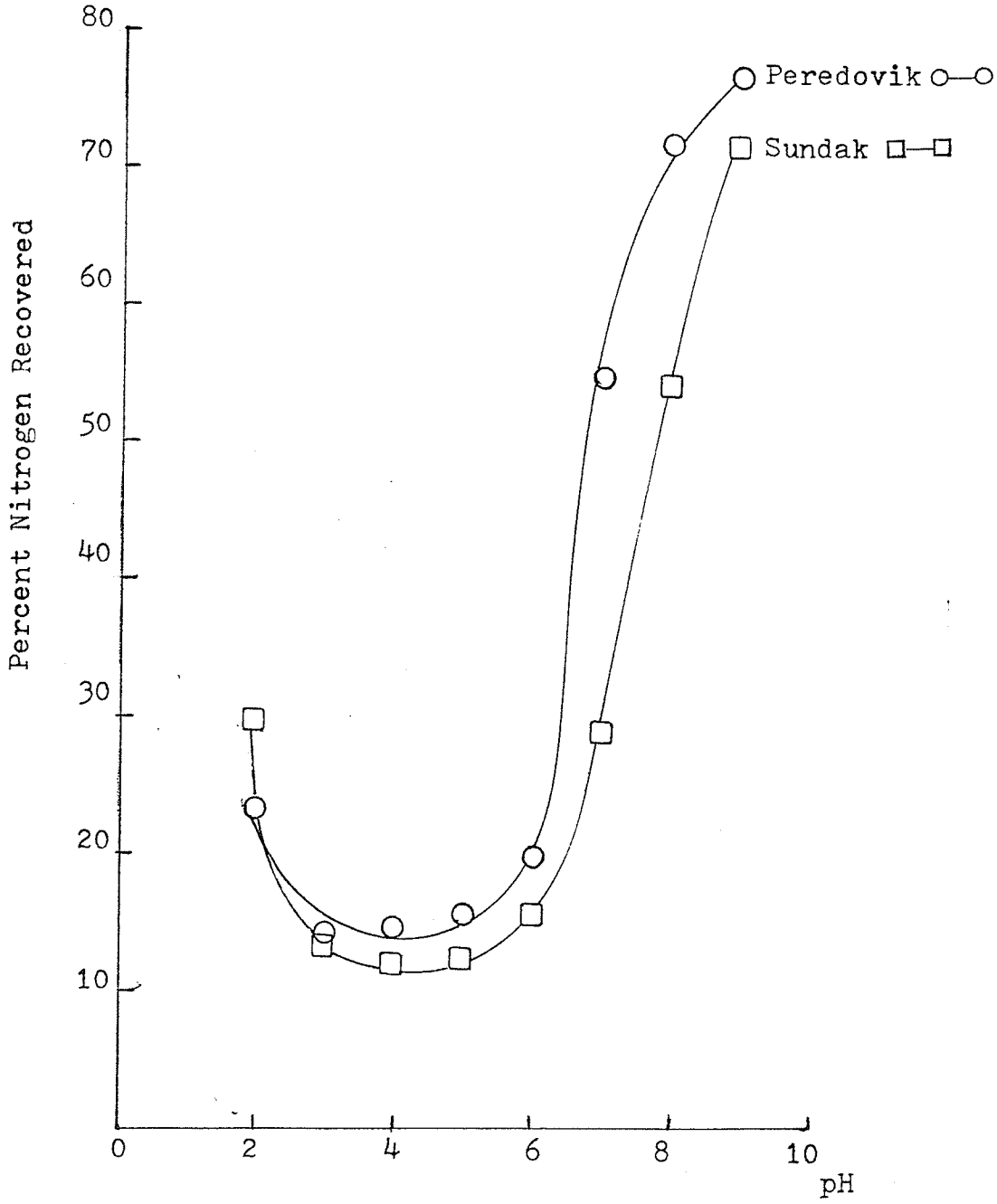


Figure 4. Nitrogen solubility of protein concentrate extracted with Methanol and Butanol Batch procedure. Variety Sundak

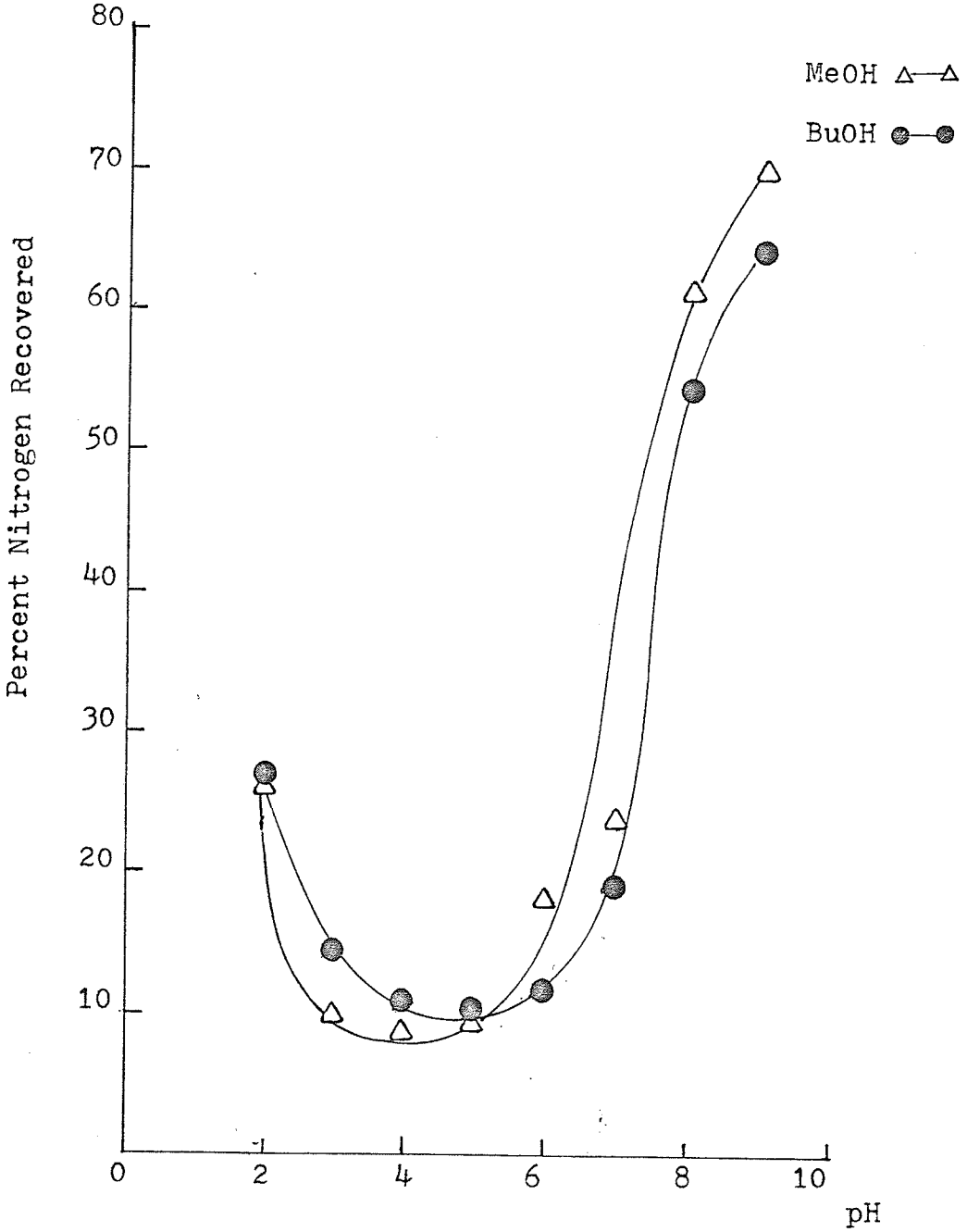


Figure 5. Nitrogen solubility of protein concentrate extracted with Butanol. Variety Sundak

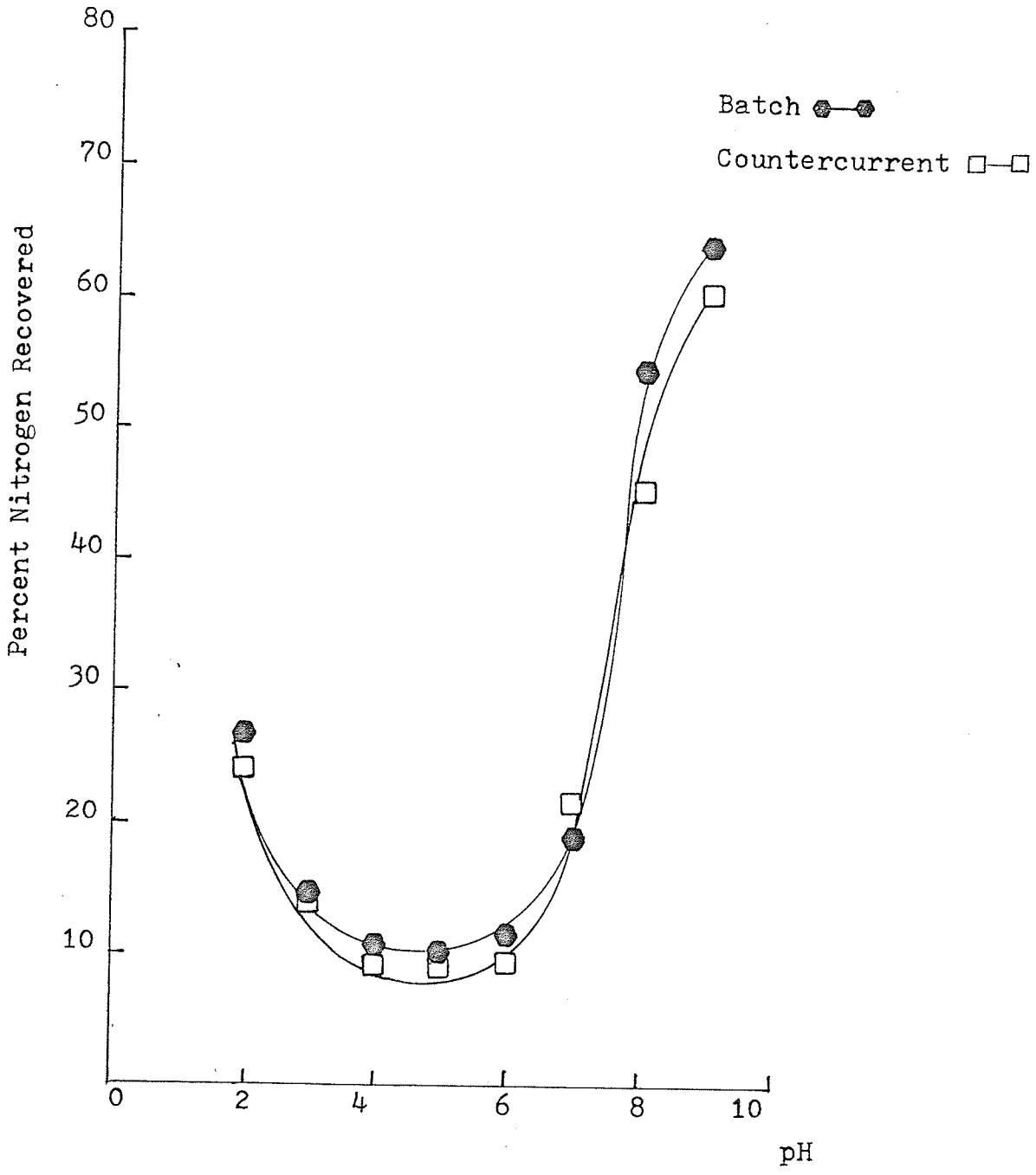


Figure 6. Nitrogen solubility of protein concentrate extracted with Methanol. Variety Sundak

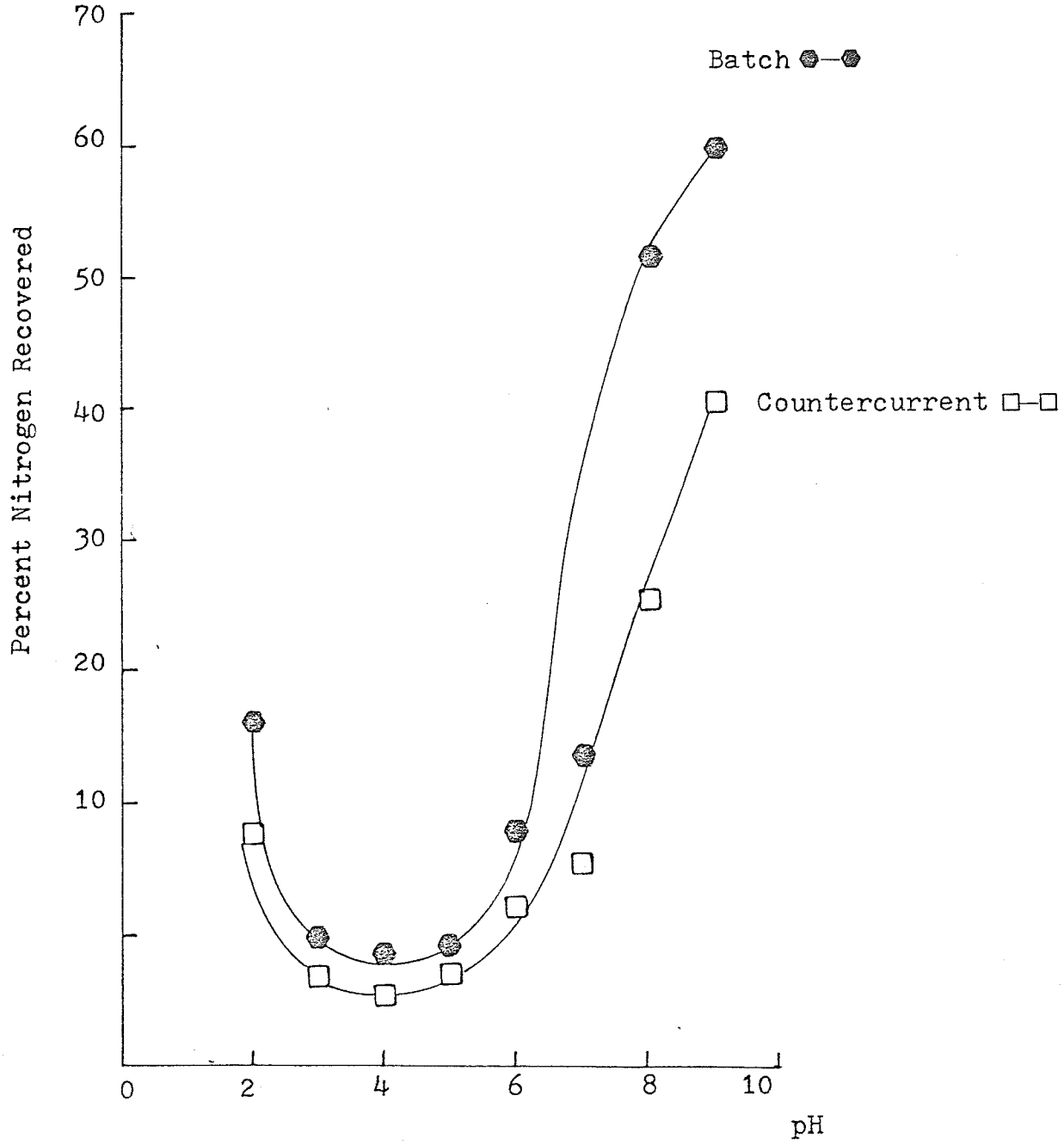


Figure 7. Nitrogen solubility of protein concentrate extracted with Methanol and Butanol Batch procedure. Variety Peredovik

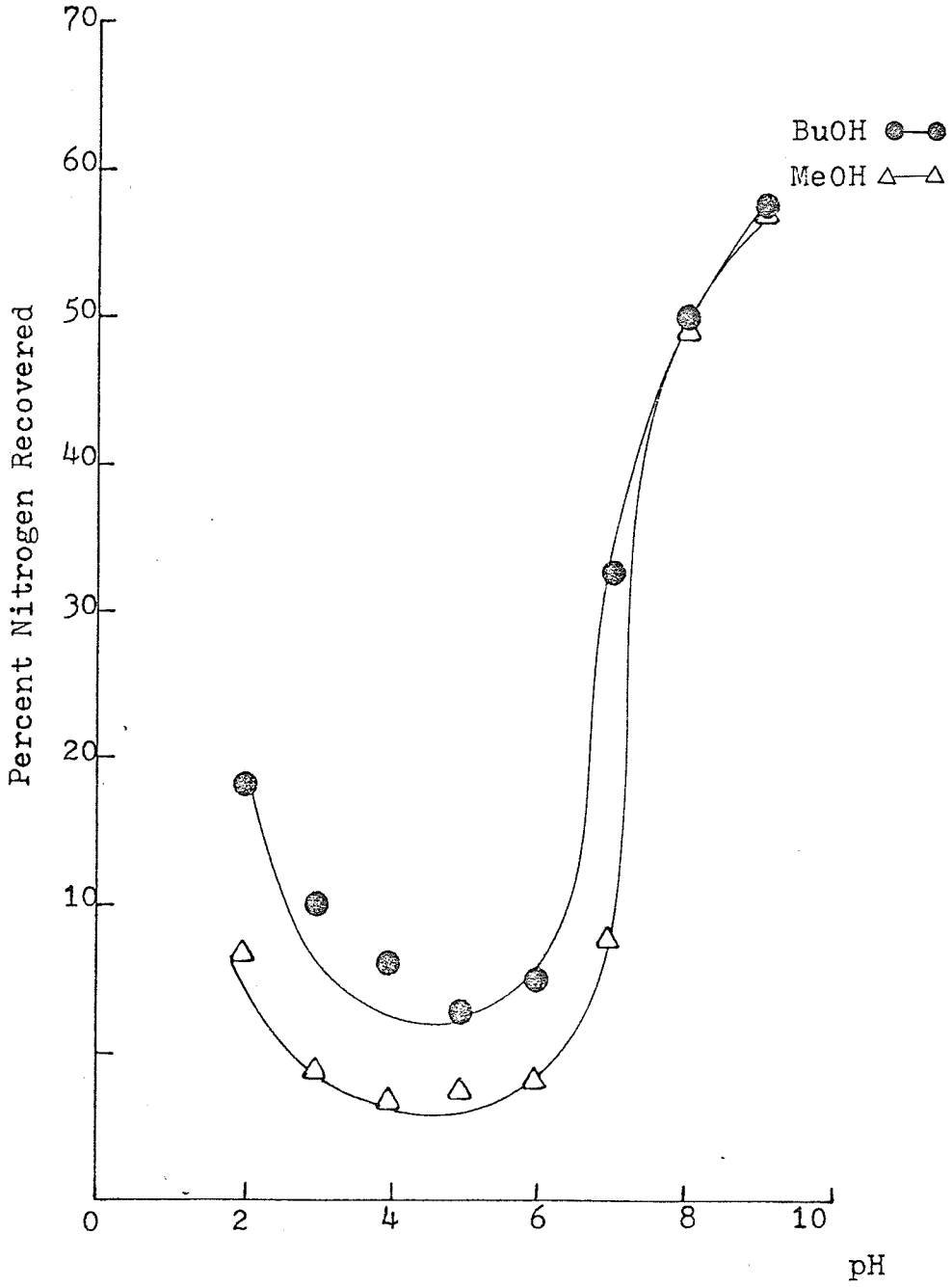


Figure 8.. Nitrogen solubility of protein concentrate extracted with Butanol. Variety Peredovik

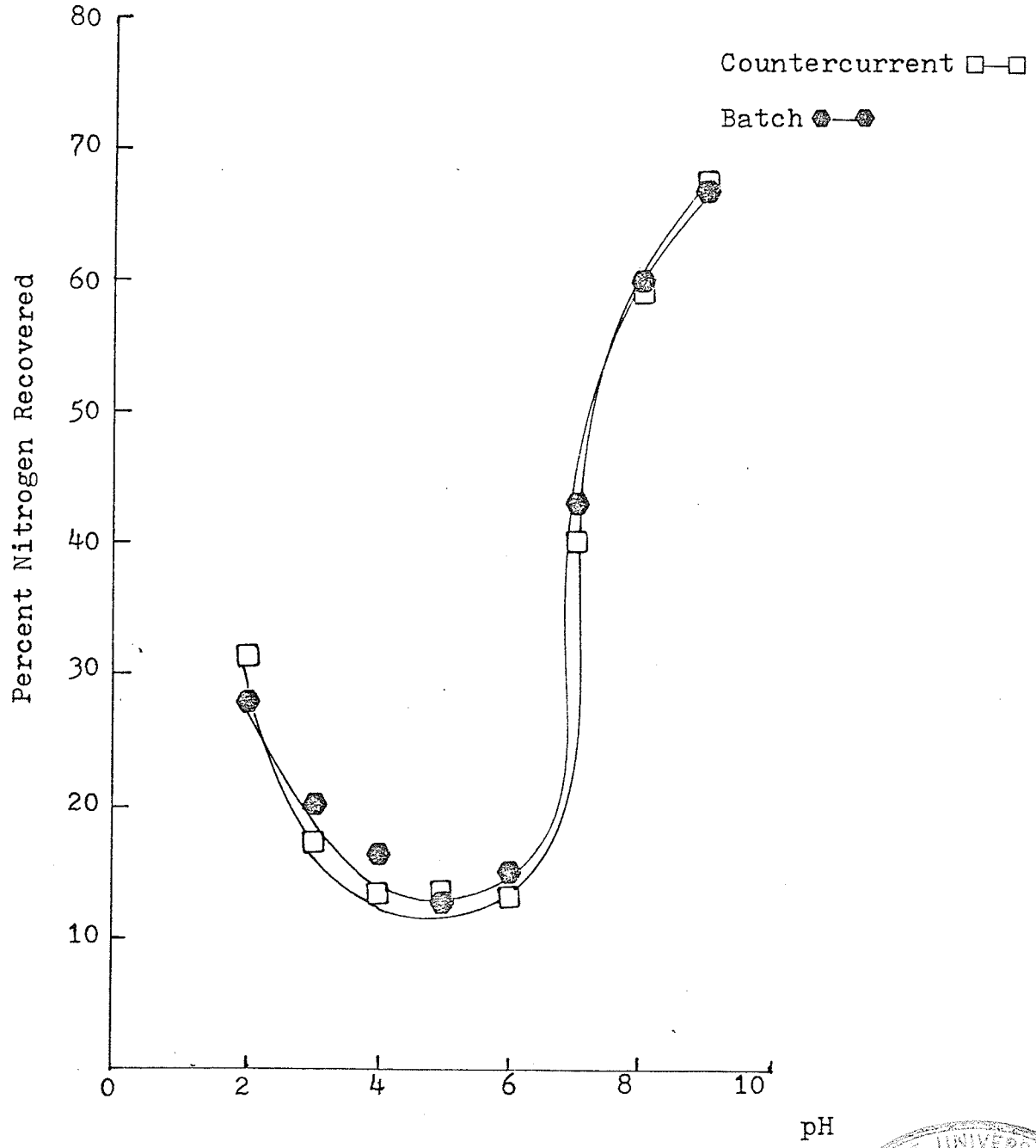
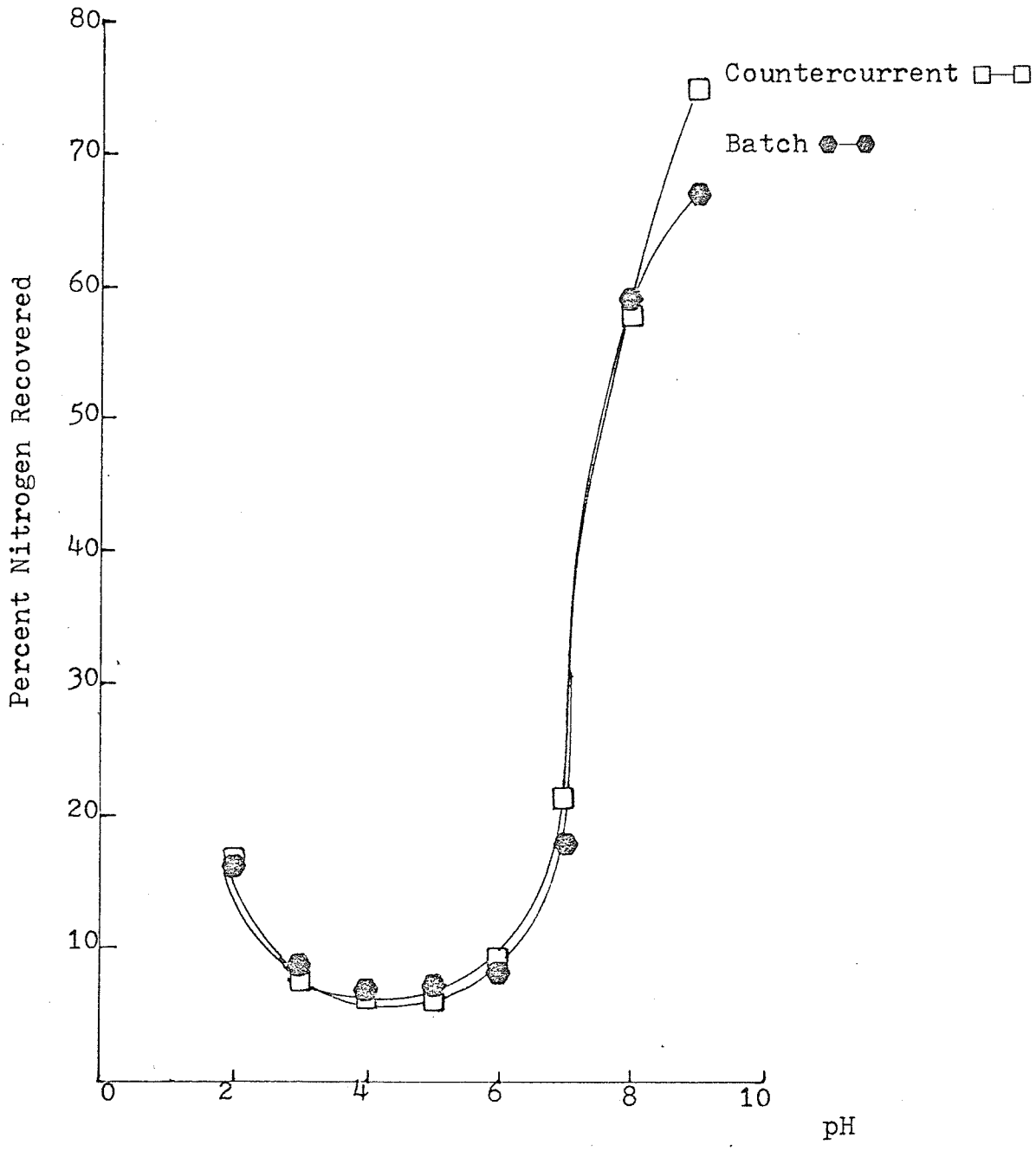


Figure 9. Nitrogen solubility of protein concentrate extracted with Methanol. Variety Peredovik



meter. Color measurements were performed on the dry samples and on flour in water slurries.

All the dry samples had a buff appearance. Extraction with acidic butanol yielded lighter protein concentrates than extraction with acidic methanol as indicated by higher L values and lower ΔE values (Table 7). This was the case for both varieties and both extraction procedures. However color measurements on dry samples are of limited value in evaluating color characteristics of protein concentrates. Such preparations would probably be added to food products and subsequently heated (cooked). Evaluation of the color on flour in water slurries submitted to heat treatment at neutral and alkaline pH is therefore more meaningful because these systems simulate actual food systems and processing conditions. Hunter values obtained on flour in water slurries (pH 5.0 to 6.5) are shown in Appendix 6 and the Hunter values determined on the same slurries after heat treatment are tabulated in Appendix 7. Comparison of the Hunter values for the slurries before and after heating suggested that heating does not increase discoloration. The appearance of the protein concentrate slurries was a light buff with Hunter values within 66 and 72 for L, -1.4 and 0 for a and 6.4 and 9.2 for b. These values compare favorably with Hunter values obtained on heated 8% wheat flour pastes which were as follows: L = 74, a = 4.2, and b = 11.8 (Hoehn et al, 1976). The protein concentrate slurries were lighter, indicated by higher

Table 7

Color Measurement on Dry Meals and Protein Concentrates

Variety	Treatment and Solvent	L 100 = white 0 = black	a pos = red neg = green	b pos = yellow neg = blue	ΔE high = more color low = less color
SUNDAK	Partially defatted Extracted with hexane (Control)	82.27d	-0.6c,d	1.5e	11.57
	Batch. Ac. BuOH	87.60b	-1.16a,b	0.53g	6.44
	Batch. Ac. MeOH	74.00f	-0.73c,d	4.73b	19.94
	Countercurrent Ac. BuOH	87.40b	-0.96b,c	0.80f	6.57
	Countercurrent Ac. MeOH	77.20e	-1.16a,b	5.30a	16.90
	Extracted with hexane (fully defatted) (Control)	82.13d	-0.53d	1.73d	11.69
	Batch. Ac. BuOH	87.43b	-1.40a	0.70f	6.57
	Batch. Ac. MeOH	83.70c	-1.16a,b	3.73c	10.20
	Directly extd. with Ac. BuOH	89.00a	-1.46a	-0.03h	5.96
PEREDOVIK	Extracted with hexane (Partially defatted) (Control)	79.90d	-0.33a	3.16d	13.97
	Batch. Ac. BuOH	87.13a	-1.13c,d	0.63f	6.87
	Batch. Ac. MeOH	76.43g	-0.70b	6.00a	17.76
	Countercurrent Ac. BuOH	86.16b	-0.86c,b	0.33g	7.88
	Countercurrent Ac. MeOH	78.00f	-0.90c,b	4.80b	15.99
	Extracted with hexane (fully defatted) (Control)	82.20c	-0.40a	2.16e	11.62
	Batch. Ac. BuOH	87.20a	-1.26d,c	0.30g	6.89
	Batch. Ac. MeOH	79.23e	-1.00c	4.53c	14.73
	Directly extd. with Ac. BuOH.	87.43a	-1.26d,c	0.33g	6.72

*Values not bearing the same subscript are significantly different, p≥0.05

L values, than the defatted meal slurries. Both solvent systems, independently of procedure, yielded similar preparations in terms of color. This was true for both varieties used in this study.

It has been well documented that discoloration of sunflower meals was accelerated under alkaline conditions. Green discolorations occurred at pH 8.0 and higher, attributed to the presence of chlorogenic acid and other phenolics (caffeic acid, iso and neo-chlorogenic acids, etc.). A green color was observed in flour in water slurries of partially and totally defatted meals when the pH was adjusted to 8. The Hunter values before heating are shown in Appendix 8 and the Hunter values of the same slurries after heating are shown in Table 8. Heating of the alkaline slurries increased discoloration slightly as evident from Hunter a and L values in Table 8. No greenness was observed in the alkaline protein concentrate slurries as indicated by a values between -2.9 and 2.8. The protein concentrate slurries were lighter in appearance than the defatted meal slurries, indicated by Hunter L values (Appendixes 6, 7 and 8, Table 8).

Both solvent systems and both extraction procedures had similar effects in terms of color as indicated by the Hunter values obtained on alkaline protein concentrate slurries. Again this was true for both varieties.

Color measurements on the alkaline and heated slurries served as a basis for investigation of the relationship

Table 8

Color Measurements on Slurries, pH 8.0, Heated up to 80°C.

Variety	Treatment and Solvent	L 100 = white 0 = black	a pos = red neg = green	b pos = yellow neg = blue	ΔE high = more color low = less color
	Control (Partially Defatted)	54.26f	-8.00b	9.1c,d	40.70
	Batch. Ac. BuOH	54.30f	-0.53e	14.26a	41.27
	Batch. Ac. MeOH	57.36d	-0.16e	12.76b	37.91
	Countercurrent Ac. BuOH	58.23c	-1.33d	9.30c	36.24
	Countercurrent Ac. MeOH	52.60g	2.50g	8.46e	41.81
SUNDAK	Control (Fully Defatted)	42.60h	-10.40a	6.73g	52.22
	Batch. Ac. BuOH	55.06e	-2.90c	8.96d	39.34
	Batch. Ac. MeOH	59.10b	2.40g	7.30f	35.23
	Non deftd. extd. Ac. BuOH	63.16a	0.56f	9.00d,c	31.40
	Control (Partially Defatted)	48.90g	-9.80b	6.70g	45.94
	Batch. Ac. BuOH	59.56c	-0.50e	8.00f	34.70
	Batch. Ac. MeOH	55.76f	2.80g	9.70a	38.94
	Countercurrent Ac. BuOH	56.20e	-1.93c	9.06c	38.21
	Countercurrent Ac. MeOH	64.10a	1.13f	8.46e	30.41
PEREDOVIK	Control (Fully Defatted)	37.10h	-13.90a	4.96h	58.18
	Batch. Ac. BuOH	58.53d	-1.00d	8.86d	35.86
	Batch. Ac. MeOH	62.03b	1.20f	9.03c	32.55
	Non-deftd. extd. Ac. BuOH	58.13d	-0.40e	9.4b	36.37

*Values not bearing the same subscript are significantly different (p 0.05).

between discoloration and certain constituents in sunflower meals and protein concentrates. This treatment was chosen because it showed most severe degrees of discoloration of all the systems investigated.

Multiple regressions were calculated. Hunter L (lightness) or ΔE (total color) values were the dependent variables. Concentration of phenolics, concentration of soluble sugars, nitrogen solubility index, and ΔH were chosen as the independent variables. As expected, it was found that phenolics contributed largely to discoloration in both varieties. Both lightness (L) and total color (ΔE) increased proportionally with increase in phenolics as shown in Tables 9 and 10. Protein denaturation (as measured by nitrogen solubility index) was the only other significant factor concerning discoloration.

On sample slurries of Sundak variety 17% of the changes in lightness (L) and 14% of changes in total color (ΔE) could be attributed to protein denaturation (Table 9). However for the Peredovik variety protein denaturation seemed to be of negligible importance. Only 1% change of lightness or total color was related to nitrogen solubility index (Table 10). The amount of sugars found in the samples did not contribute to discoloration.

The results of the regression analysis indicated the importance of phenolics, suggesting that discoloration could be predicted by simply determining linear regression between

phenolics and discoloration. In Figures 10, 11 and 12, Hunter readings L, a and ΔE were plotted against phenolic concentration. Hunter a values and phenolics were highly correlated for both varieties. The linear correlation between phenolics and lightness (L) or total color (ΔE) was high for the Peredovik variety but moderate for the samples of Sundak variety.

Table 9

Multiple Regression for Color Measurements

Condition (4). Variety Sundak

Variable	Multiple r	r^2	r^2 Change	Simple r	B	β	F*
Dependent variable L							
Phenolics	0.7685	0.59059	0.59059	-0.7685	-6.21285	-1.4751	18.634a
NSI	0.87241	0.76111	0.17052	-0.5108	0.70473	1.0290	14.085a
Sugars	0.87323	0.76252	0.00142	-0.5789	-0.20009	-0.1488	0.342
Δ H	0.87467	0.76504	0.00252	-0.6213	-0.42320	-0.1142	0.236
(Constant)					47.4530		
Dependent Variable Δ E							
Phenolics	0.76373	0.58329	0.58329	0.76373	5.678573	1.32563	13.36a
NSI	0.85253	0.72681	0.14352	0.52145	-0.686775	-0.98596	11.50a
Δ H	0.85349	0.72845	0.00164	0.63271	0.707167	0.18757	0.56
Sugars	0.85746	0.73524	0.00679	0.58314	0.277376	0.20280	0.56
(Constant)					46.53094		

*df = N-K-1 = 22; where N = sample size; K = number of independent variables

[a] Significant at p = 0.01

Table 10

Multiple Regression for Color Measurements

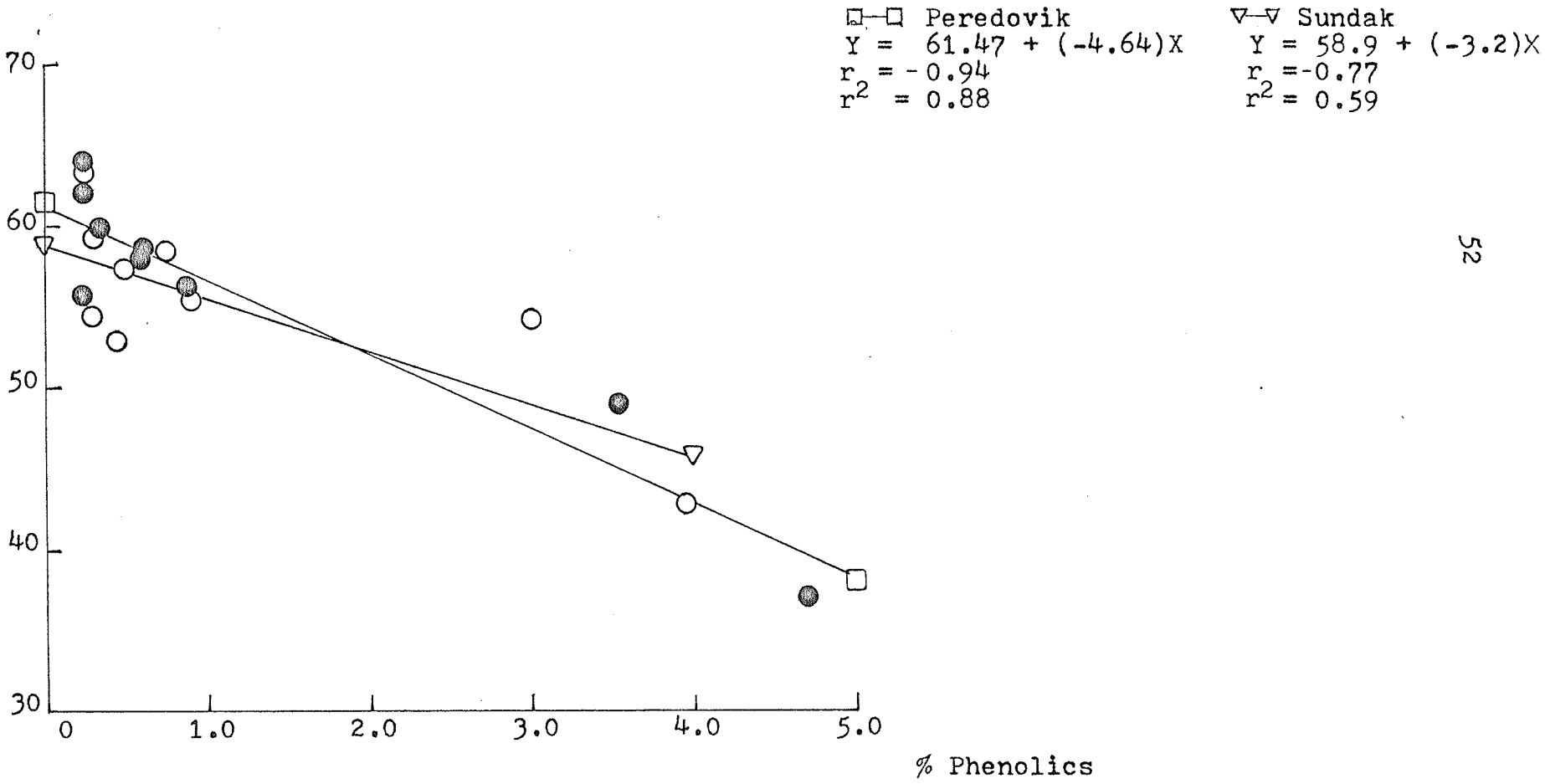
Condition (4). Variety Peredovik

Variable	Multiple r	r^2	r^2 Change	Simple r	B	β	F*
Dependent Variable L							
Phenolics	0.94053	0.88460	0.88460	-0.94053	-4.413794	-0.89518	41.71a
Δ H	0.94225	0.88783	0.00323	-0.75138	-1.205609	-0.16638	1.83
NSI	0.94787	0.89846	0.01062	-0.75952	0.2727177	0.45259	4.26b
Sugar	0.95201	0.90632	0.00786	-0.83584	-0.5789869	-0.33871	1.85
(Constant)					57.49588		
Dependent Variable ΔE							
Phenolics	0.94184	0.88706	0.88706	0.94184	4.722706	0.92740	45.93a
NSI	0.94429	0.89169	0.00463	0.75266	-0.2844743	-0.45710	4.45b
Δ H	0.94977	0.90207	0.01038	0.74545	1.221458	0.16321	1.81
Sugar	0.95325	0.90868	0.00661	0.82923	0.5484527	0.31065	1.60
(Constant)					37.27996		

*df = (N-k-1) = 22; where N = total number of cases; k = number of independent variables.

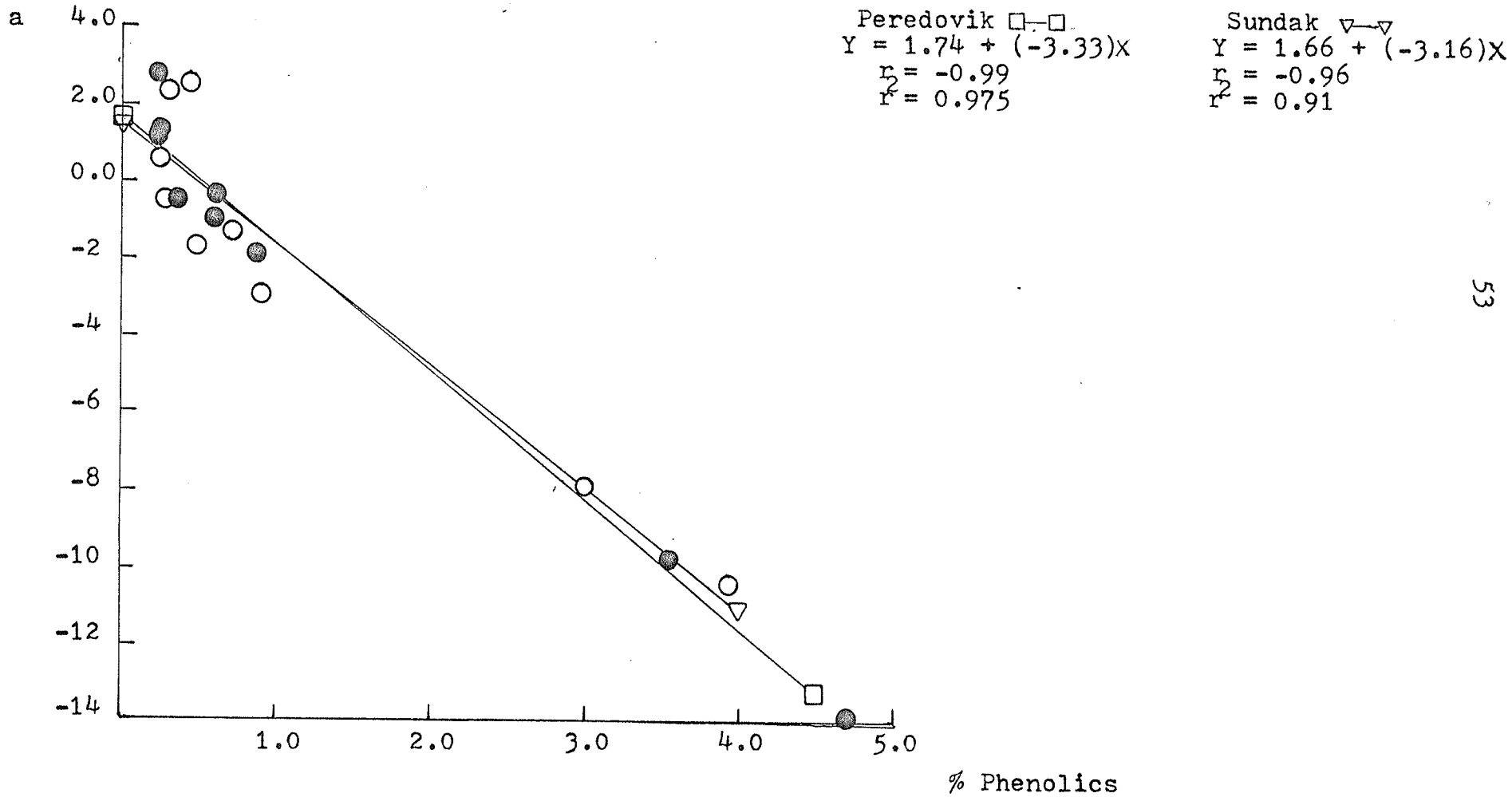
a - Significant at p = 0.01
b - Significant at p = 0.05

Figure 10. Effect of Phenolics on Lightness (L)*



*Condition 4

Figure 11. Effect of Phenolics on Values of a*

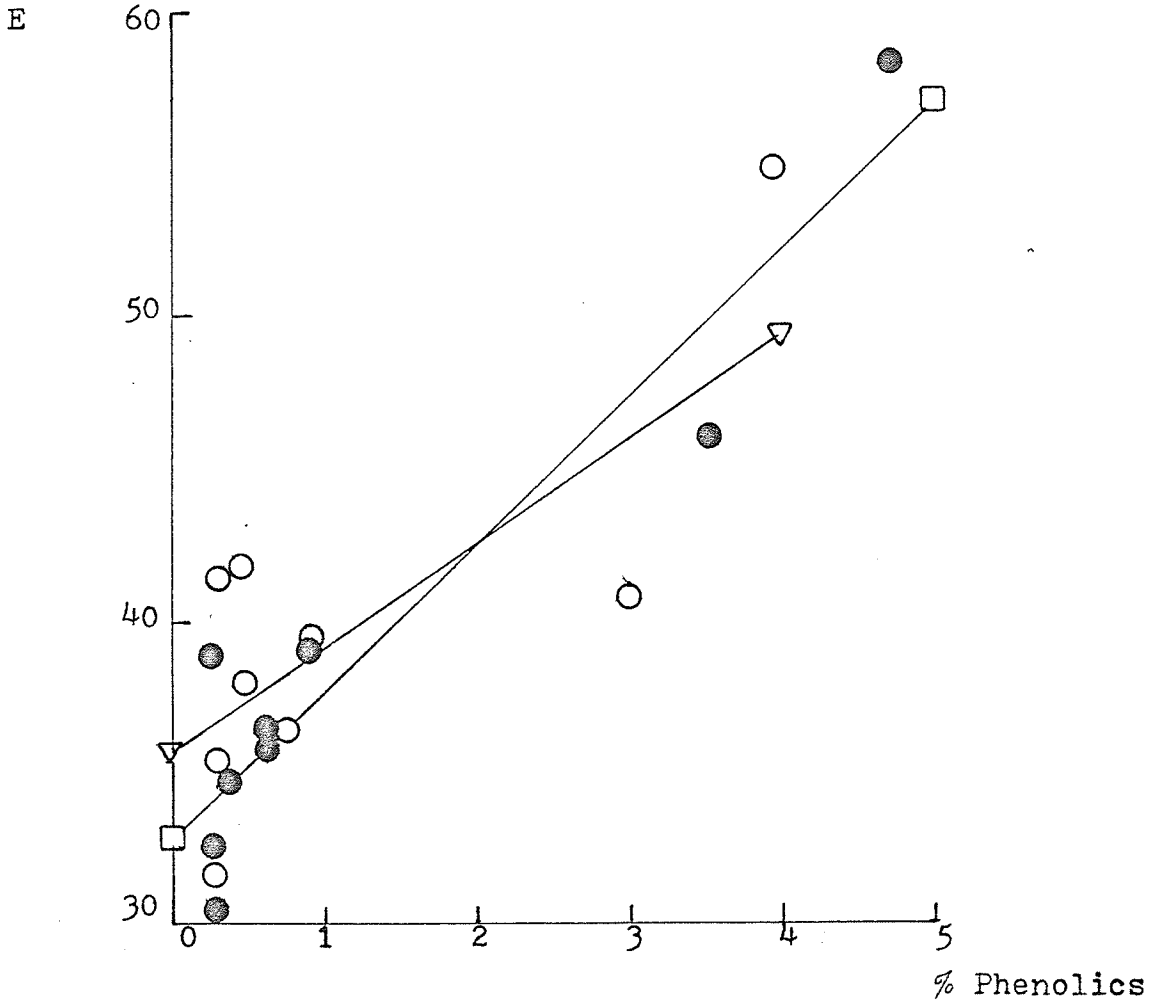


*Condition 4

Figure 12 . Effect of Phenolics on Total Color Change (ΔE)*

Peredovik □—□
Y = 32.94 + 4.8X
r_2 = 0.94
r^2 = 0.89

Sundak ▽—▽
Y = 35.8 + 3.27X
r_2 = 0.76
r^2 = 0.58



*Condition 4

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

The present study investigated the effects of acidic butanol and acidic methanol in the preparation of sunflower protein concentrates. Several criteria were used to assess the potential of acidic butanol or acidic methanol as solvents in preparing protein concentrates from Peredovik and Sundak sunflower kernels.

These criteria were:

1) Efficiency in removing phenolics and 80% ethanol soluble sugars.
2) Protein losses, loss of lysine and isoleucine
3) Protein denaturation
4) Magnitude of discoloration

Both solvent systems were equally effective in removing phenolics. Acidic methanol was more effective in removing sugars than acidic butanol. Protein losses were similar regardless of the solvent system used. The quality of the resulting concentrates differed. Protein quality was lower when acidic methanol was employed, as measured by protein denaturation (NSI, ΔH, and nitrogen solubility profiles). Regardless of which solvent system was used, the quality of the concentrates in terms of color was similar. Both systems yielded an acceptable product.

Two procedures were followed. These were a batch procedure or a countercurrent procedure. The use of less solvent was the obvious advantage of the countercurrent scheme. Otherwise there were no advantages in using one system rather than the other.

Phenolics were the main cause of discoloration of the meals and protein concentrates, as indicated by the statistical analyses of the data on color measurements. However the protein denaturation (measured by NSI) contributed to the variation of lightness (L) and total color (E). One explanation could be that, the denaturated protein has more amino acid residues exposed. These can react with other compounds present in the slurry, e.g. sugars (Maillard reaction), giving colored products. Based on the data presented in the results and discussion there were no discernable differences between acidic butanol and acidic methanol. Other factors must be considered. If defatted meals are used as the starting material, acidic methanol would be the solvent of choice. Methanol was more easily removed from the resulting concentrates than butanol. Butanol also effectively removes lipids, hence crushed sunflower kernels can be used as the starting material. Phenolics are extracted with the oil and further studies should be undertaken to determine their ease of extraction from the oil.

Further study is required on the effect of different alcohols in terms of protein denaturation. It appears that

when there is little water present in the solvent system the effect of alcohol on protein denaturation may be inversely proportional to the chain length.

Evaluation of the functional properties of protein concentrates obtained with the methods used in this study, along with incorporation into food systems would provide the final proof on the feasibility of such concentrates as food ingredients. One possible application of sunflower protein concentrates would be to supplement high caloric diets. Such diets are still predominant in developing countries, including Mexico, where cereals and pulses constitute a big portion of the daily diet.

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Appendix 1

Composition of Meal and Protein Concentrates

Variety	Constituent	Partially defatted meal (Control)	Extracted with Acidic MeOH Batch	Extracted with Acidic MeOH C.C.	Extracted with Acidic BuOH Batch	Extracted with Acidic BuOH C.C.	Totally defatted meal (Control)	Extracted with Ac. MeOH	Extracted with Acidic BuOH	Non-defatted directly extracted with Acidic BuOH
SUNDAK	% Protein (N x 6.25)	44.3	47.2	52.1	63.9	62.6	54.9	67.3	65.5	63.2
	% Sugars (soluble in 80% EtOH, as glucose)	10.8	0.37	0.96	6.11	5.86	11.76	0.36	6.85	4.36
	% Phenolics (as Chlorogenic acid)	3.0	0.49	0.45	0.30	0.74	3.95	0.29	0.90	0.27
	% Yield in Proteins	100	83.5	90.8	89.7	91.9	97.0	83.9	88.7	82.1
	% Yield of total solids (100% ground seeds)	61.8	48.3	47.7	38.4	40.2	48.4	34.0	37.0	35.5
PEREDOVIK	% Protein (N x 6.25)	36.9	42.0	42.0	62.5	59.8	50.0	62.8	61.3	58.8
	% Sugars (soluble in 80% EtOH, as glucose)	11.79	0.39	0.39	4.92	7.63	13.0	0.44	6.45	3.86
	% Phenolics (as Chlorogenic acid)	3.53	0.25	0.26	0.33	0.87	4.70	0.25	0.61	0.61
	% Yield in Proteins	100	86.9	86.6	89.6	90.7	97.2	85.5	89.1	76.6
	% Yield of total solids (100% ground seeds)	52.4	40.0	39.9	27.7	29.3	37.5	26.4	28.0	27.0

Appendix 2

Amino Acid Composition of Sunflower Protein Concentrates
Variety Sundak

Amino Acid*	Ptly. Deftd. Ac. BuOH Batch	Ptly. Deftd. Ac. MeOH Batch	Ptly. Deftd. Ac. BuOH C.C.[1]	Ptly. Def Ac. MeOH C.C.
Lysine	1.461	1.139	1.430	1.228
Histidine	1.002	0.823	0.955	0.940
Ammonia	1.062	0.798	1.081	0.947
Arginine	3.400	2.834	3.845	3.495
Aspartic Acid	3.547	2.878	3.798	3.467
Threonine	1.394	1.183	1.411	1.322
Serine	1.438	1.172	1.513	1.382
Glutamic Acid	7.734	6.127	8.330	7.483
Proline	1.612	1.362	1.742	1.598
Glycine	2.182	1.761	2.317	2.092
Alanine	1.608	1.411	1.765	1.644
Cystine	1.026	0.951	1.046	1.028
Valine	2.185	1.883	2.424	2.214
Methionine	1.221	1.422	1.228	1.359
Isoleucine	1.785	1.491	1.873	1.714
Leucine	2.561	2.158	2.774	2.527
Tyrosine	0.597	0.647	0.955	0.832
Phenylalanine	1.675	1.545	1.969	1.808

*% A.A.

[1]Countercurrent

Appendix 3

Amino Acid Composition of Sunflower Protein Concentrates
Variety Sundak

Amino Acid*	Fully Deftd. Ac. BuOH Batch	Fully Deftd. Ac. MeOH Batch	Directly Extd. Ac. BuOH Batch
Lysine	1.676	1.802	1.642
Histidine	1.244	1.318	1.225
Ammonia	1.261	1.371	1.262
Arginine	4.442	4.559	4.456
Aspartic Acid	4.518	4.709	4.424
Threonine	1.696	1.873	1.671
Serine	1.779	1.924	1.765
Glutamic Acid	9.776	10.518	9.633
Proline	2.017	2.118	1.874
Glycine	2.658	2.876	2.674
Alanine	1.996	2.143	1.977
Cystine	1.358	1.659	1.857
Valine	2.817	2.968	2.786
Methionine	1.532	1.631	1.397
Isoleucine	2.233	2.388	2.171
Leucine	3.202	3.408	3.213
Tyrosine	0.950	0.881	1.212
Phenylalanine	2.325	2.236	2.351

*% A.A.

[1]Countercurrent

Appendix 4

Amino Acid Composition of Sunflower Protein Concentrates
Variety Peredovik

Amino Acid*	Ptly. Deftd. Ac. BuOH Batch	Ptly. Deftd. Ac. MeOH Batch	Ptly. Deftd. Ac. BuOH C.C.[1]	Ptly. Deftd. Ac. MeOH C.C.	Directly extd. Ac. BuOH Batch
Lysine	1.807	1.064	1.561	1.005	1.633
Histidine	1.309	0.727	1.090	0.704	1.123
Ammonia	1.354	0.678	1.156	0.651	1.103
Arginine	4.831	2.533	3.759	2.437	3.899
Aspartic Acid	4.793	2.475	3.761	2.431	3.950
Threonine	1.761	1.003	1.393	1.012	1.551
Serine	1.903	1.031	1.638	1.021	1.623
Glutamic Acid	10.520	5.384	8.594	5.084	8.465
Proline	2.186	1.191	1.609	1.117	1.757
Glycine	2.824	1.568	2.337	1.516	2.455
Alanine	2.121	1.226	1.670	1.206	1.773
Cystine	1.172	0.647	0.769	0.694	0.932
Valine	2.922	1.628	2.370	1.602	2.510
Methionine	1.463	0.971	1.071	1.079	1.124
Isoleucine	2.325	1.263	1.878	1.246	1.956
Leucine	3.429	1.909	2.793	1.878	2.948
Tyrosine	1.221	0.713	0.888	0.658	0.944
Phenylalanine	2.472	1.341	1.798	1.315	1.853

*% A.A.

[1]Countercurrent

Appendix 5

Calculation of % Compounds Removed

$$\frac{C - (A \times B)}{C} \times 100 = D$$

Where

A. % of compound in protein concentrate

B. % yield in solids

C. % of compound in control meal

D. % of compound removed

Example.

A = 0.3%

B = 27.7%

C = 3.5%

$$\frac{3.5 - (0.3 \times 0.277)}{3.5} \times 100 = 97.62\% \text{ of compound removed}$$

Appendix 6

Color Measurements* on Slurries Condition 1

Variety	Treatment and Solvent	L 100 = White 0 = black	a pos = red neg = green	b pos = yellow neg = blue	ΔE
	Control (Partially Defatted)	62.4h	-1.03a	6.5d	31.68
	Batch. Ac. BuOH	71.37c	-1.40a	7.2b	22.96
	Batch. Ac. MeOH	65.57g	-1.10a	9.23a	29.07
	Countercurrent. Ac. BuOH	69.53e	-0.87a	7.1b	24.73
	Countercurrent. Ac. MeOH	66.23f	-0.93a	9.0a	28.37
SUNDAK	Control (Fully Defatted)	60.47i	1.40b	6.7c,d	33.71
	Batch. Ac. BuOH	70.43d	-0.9a	7.13b	23.86
	Batch. Ac. MeOH	72.07b	-1.17a	6.97c,b	22.22
	Directly extd. with Ac. BuOH	72.80a	-1.3a	7.07b	21.53
	Control (Partially Defatted)	63.67g	0.53f	7.03a	30.54
	Batch. Ac. BuOH	72.2a	-1.1b	6.97a,b	22.1
	Batch. Ac. MeOH	68.43e	-0.63d,e	6.57c,d	25.72
	Countercurrent. Ac. BuOH	67.10f	-0.4e	6.6c,d	27.05
	Countercurrent. Ac. MeOH	69.80d	-1.03b,c	6.50d,e	24.36
PEREDOVIK	Control (Fully Defatted)	61.83h	0.9g	6.4e	32.29
	Batch. Ac. BuOH	71.73b	-0.87c,d	6.87b	22.53
	Batch. Ac. MeOH	67.27f	-0.5e	6.70c	26.90
	Directly extd. with Ac. BuOH	70.90c	-1.4a	6.4e	23.26

*Values not bearing the same subscript are significantly different at p 0.05.

Appendix 7

Color Measurements on Slurries Condition 2

Variety	Treatment and Solvent	L 100 = white 0 = black	a pos = red neg = green	b pos = yellow neg = blue	ΔE high = more color low = less color
	Control (Partially Defatted)	59.83e	1.0d	5.77e	34.20
	Batch. Ac. BuOH	70.87b	-1.40a,b	7.07c	23.42
	Batch. Ac. MeOH	65.23d	-1.43a	9.63a	29.49
	Countercurrent. Ac. BuOH	71.67a	-0.90a,b,c	6.50d	22.52
	Countercurrent Ac. MeOH	68.43c	-1.2a,b,c	9.13b	26.27
SUNDAK	Control (Fully Defatted)	58.57f	1.0d	6.30d	35.52
	Batch. Ac. BuOH	71.2a	-0.83b,c	6.37d	22.96
	Batch. Ac. MeOH	70.50b	-1.0a,b,c	7.00c	23.76
	Directly extd. with Ac. BuOH	68.8c	-0.73c	7.27c	25.49
	Control (Partially Defatted)	63.0g	0.87e	7.33a	31.27
	Batch. Ac. BuOH	70.23a	-1.1a	5.83g	23.83
	Batch. Ac. MeOH	67.30d	-0.90a,b	6.83c	26.88
	Countercurrent. Ac. BuOH	66.97d	0.0d	6.23f	27.14
	Countercurrent. Ac. MeOH	68.93b	-0.80b	6.47d	25.21
PEREDOVIK	Control (Fully Defatted)	58.33h	1.4f	6.30e,f	35.87
	Batch. Ac. BuOH	67.97c	-0.53c	6.37d,e	26.15
	Batch. Ac. MeOH	65.5e	-0.10d	7.0b	28.70
	Directly extd. with Ac. BuOH	63.73f	0.1d	7.27a	30.34

*Values not bearing the same subscript are significantly different, p 0.05.

Appendix 8

Color Measurements* on Slurries. Condition 3

Variety	Treatment and Solvent	L 100 = white 0 = black	a pos = red neg = green	b pos = yellow neg = blue	ΔE
	Control (Partially Defatted)	56.60e	-1.2b	15.50b	39.47
	Batch. Ac. BuOH	54.90f	-0.53c	18.83a	41.27
	Batch. Ac. MeOH	54.77f	3.20f	10.47f	40.10
	Countercurrent. Ac. BuOH	61.37b	-0.67b,c	13.03d	36.24
	Countercurrent. Ac. MeOH	52.67g	2.57e	8.90h	41.81
SUNDAK	Control (Fully Defatted)	50.33h	-6.67a	14.20c	45.40
	Batch. Ac. BuOH	59.77c	0.9d	14.10c	36.07
	Batch. Ac. MeOH	58.03d	3.5f	9.73g	36.81
	Directly extd. with Ac. BuOH	65.13a	-0.07c	11.73e	30.19
	Control (Partially Defatted)	60.87c,d	-2.13c	17.77b	36.39
	Batch. Ac. BuOH	60.50d	-3.40b	19.70a	37.64
	Batch. Ac. MeOH	62.63a	0.80g	6.97h	31.57
	Countercurrent. Ac. BuOH	58.40f	0.00e	15.33d	37.77
	Countercurrent. Ac. MeOH	61.73b	1.00g	11.60g	33.45
PEREDOVIK	Control (Fully Defatted)	51.40g	-6.97a	16.87c	45.21
	Batch. Ac. BuOH	61.07c	0.27f	14.63e	35.00
	Batch. Ac. MeOH	61.90b	0.40f	11.40g	33.20
	Directly extd. with Ac. BuOH	58.93e	-0.60d	12.43f	36.31

*Values not bearing the same subscript are significantly different, p 0.05.